RELATEDNESS, GENETIC DIVERSITY AND THE BEHAVIOUR OF BREEDING GREY SEALS, *HALICHOERUS GRYPUS*

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Contents

Declarations

I, Veronica Poland, hereby certify that this thesis, which is approximately 62 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

The understanding of animal behaviour may often be enhanced by the inclusion of genetic information. In this study, I examined how the behaviour of grey seals in the North Rona breeding colony varied with their relatedness and genetic diversity. In order to calculate relatedness and genetic diversity estimates and to assign parentage, over 600 individuals were genotyped at 11 microsatellite loci.

Mothers were predicted to pup near relatives in the colony. However, kin clustering was observed in one region of the colony only. This suggests that kin segregation within the colony is not frequent and may require a particular combination of topography, density and site use. I also predicted that mothers with high genetic diversity and average relatedness to the colony would pup in areas with preferred topographic characteristics, early in the season. Patterns of breeding females were examined over four years using spatial autocorrelation measures. There was some evidence that females with higher genetic diversity pupped in locations with high female density, which may represent areas of good habitat. However, no relationship was detected between average relatedness to the colony and habitat.

Individuals may display less aggression towards kin to increase their inclusive fitness. However, there was little evidence of kin-biased aggression reduction between female grey seals. Instead, higher aggression rates were generally observed when females were near pools and at higher density. In one year, females with higher genetic diversity also tended to be more aggressive, suggesting they may have greater competitive ability. There was also no evidence that males or females mated or associated with members of the opposite sex that they were least related to. Paternity analysis suggested that in some locations around 60% of pups could be assigned parentage and that the sampling regime may affect the proportion of pups assigned fathers.

Chapter 1: General introduction

1.1 Using genetics to understand social behaviour

The study of social behaviour encompasses all interactions between conspecifics, such as competition, family dynamics and mating behaviour. Social behaviours can range from brief interactions between individuals that live an almost solitary existence to complex social behaviours between group-living organisms. Our understanding of these social behaviours can often be enhanced by the inclusion of genetic information about individuals (Hughes 1998). For example, the genetic relatedness between individuals can be important in explaining the evolution of certain behaviours such as cooperation or aggression reduction (reviewed in Holmes 1988; Waldman 1988; Komdeur & Hatchwell 1999). Furthermore, genetic analysis has revealed information about mate choice and parentage, leading to a greater understanding of breeding systems (Reynolds 1996; Hughes 1998; Tregenza & Wedell 2000; Griffiths et al. 2002). Finally, an individual's genetic diversity, or level of inbreeding, may give an indication of quality, which is likely to affect social behaviour (Crnokrak & Roff 1999; Keller & Waller 2002).

1.1.1 Kin selection

The theory of kin selection was developed in 1964 by Hamilton in two seminal papers in which he outlined how the existence of altruistic and cooperative behaviour could be explained by taking into consideration the genetic relatedness of individuals (Hamilton 1964). Kin selection works on the principle that, since related individuals share genes that are identical by descent, an individual that increases the fitness of relatives will increase the number of its genes in the population. Thus, by helping relatives, an individual can increase its inclusive fitness, defined as the lifetime reproductive output of an individual plus the individual's effect on the reproductive output of its relatives, weighted by their relatedness (Hamilton 1964). Inclusive fitness can be broken down into direct fitness, which individuals gain from their own reproductive success, and indirect fitness, which individuals gain from the reproductive success of relatives. Generally, kin-biased behaviours are selected for if $rb - c > 0$, where r is the relatedness of the pair, b is the benefit to the recipient and c is the cost to the donor. For each behaviour, the costs and benefits will vary depending on the situation and the identity of the interacting pair.

Kin selection theory has been extremely useful in understanding the evolution of many social behaviours. This is especially true for eusocial insects, where the prevalence of altruistic behaviour has been explained in terms of the evolutionary consequence of the haplodiploid system (reviewed in Hughes 1998). Kin selection may also explain how cooperative breeding behaviour has evolved in some vertebrate species (Komdeur 1994; Russell & Hatchwell 2001; Baglione et al. 2003). Additionally, reduced aggression towards neighbouring kin in territorial animals may be explained using inclusive fitness theory (Brown & Brown 1993; Mappes et al. 1995; Griffiths & Armstrong 2002). Kin-biased behaviours, such as grooming and food sharing, are also important features of primate social groups (reviewed in Schino 2001; Isbell &Young 2002).

However, the association of kin does not necessarily indicate that indirect fitness benefits are important, even when behaviour may appear altruistic (Griffin & West 2002). Specifically, in some vertebrate cooperative breeding systems, philopatry leads to kin remaining in the natal territory or group where they help to raise offspring. However, helper investment may not be correlated with the degree of kinship, and some individuals may be unrelated to the offspring they help to rear (Dunn et al. 1995; Clutton-Brock et al. 2000). Instead, individuals may help because they are unable to find appropriate breeding sites of their own and gain direct fitness benefits by enhancing their mating opportunities or gaining access to food and protection on the territory (Dunn et al. 1995; Magrath & Whittingham 1997; Griffin & West 2002). Similarly, protection of kin-based social groups may involve cooperation or appear altruistic but may not have evolved though kin selection. However, individuals may gain direct fitness benefits from the behaviour, such as increased survivorship or access to resources (Grinnell et al. 1995; Clutton-Brock et al. 1999; Van Horn et al. 2004).

Another explanation for cooperative behaviour, that does not rely on kinship, is reciprocal altruism (Trivers 1971). Individuals may employ a tit-for-tat strategy, such that the decision to cooperate is based on recent experience; individuals reciprocate to cooperators but not to cheats (Axelrod & Dion 1988). Apart from humans, few studies have found reciprocity can successfully explain the evolution and maintenance of cooperative behaviour (e.g. Wilkinson 1984). However, Olendorf et al. (2004) recently found evidence that reciprocity maintains cooperative nest defence behaviour in some populations of red-winged blackbirds *Agelaius phoeniceus*.

For kin selection to occur, one prerequisite is that relatives interact regularly. Limited dispersal may result in high relatedness among interacting individuals (Hamilton 1964). However, this increases competition between relatives, decreasing the likelihood of altruistic behaviour (Queller 1992; West et al. 2001). If competitors are related to each other, then there will be no inclusive fitness benefits because helping one relative will be at the cost of another. For example, the level of aggression shown by males fighting for access to females in fig wasp taxa was not correlated to kinship because, within a fruit, all competitors were highly related (West et al. 2001).

1.1.2 Kin recognition

If individuals interact with both kin and non-kin they must be able to assess relatedness to gain inclusive fitness benefits. This is obviously not a mental concept that animals possess but rather an ability to alter behaviour based on the degree of relatedness. There are several mechanisms that have been proposed to explain how animals distinguish relatives (Holmes 1988; Waldman 1988). Often, animals use association, or familiarity with others, as a guide to relatedness. For instance, parents may recognise offspring based on their presence in the nest and nest mates may treat each other as siblings (Miño & Tang-Martínez 1999; Komdeur et al. 2004). Alternatively, individuals may use spatial cues, such as presence in the territory, as a basis for assigning relatedness (Komdeur & Hatchwell 1999). More complicated behavioural rules-of-thumb may also be employed to distinguish relatives. For example, savannah baboons *Papio cynocephalus* may use age, residence patterns and mating behaviour to recognise paternal kin (Buchan et al. 2003; Smith et al. 2003).

Another mechanism that animals use to identify kin is phenotype matching (Waldman 1988; Komdeur & Hatchwell 1999). Here, individuals compare the phenotype of others to themselves or a close relative; those with similar phenotypes are to be assumed to be related. Thus, unfamiliar related individuals can recognise each other when they interact and can alter their behaviour to maximise inclusive fitness. The phenotypes used for matching can be quantitative or qualitative and assessment of kinship may use a combination of phenotypic cues. For example, both chemical and visual cues are thought to be important in discriminating kin in rainbow fish, *Melanotaenia eachamensis* (Arnold 2000). Additionally, birds may use song to differentiate between

kin and non-kin (Price 1999). Odour also plays an important role in kin recognition in some species (Arcaro & Eklund 1999; Bull et al. 2001; Mateo 2002). Odour cues are thought to be based on variation in the major histocompatibility complex (MHC), known as the human leukocyte antigen (HLA) in humans (Penn & Potts 1998). These may affect odour by influencing microbial flora or concentrations of volatile acids in urine (Singer et al. 1997).

1.1.3 Mate choice and inbreeding

The ability to recognise kin is important, not only to inclusive fitness theory, but also when choosing mates. Unless individuals of at least one sex disperse from the natal site, a kin recognition system is necessary to avoid mating with kin (Pusey & Wolf 1996). Mating with relatives (inbreeding) can reduce the survival and fecundity of offspring. This decrease in fitness, known as inbreeding depression, has been well documented in wild and captive populations of plants and animals (Pusey & Wolf 1996; Crnokrak & Roff 1999; Keller & Waller 2000).

Inbreeding reduces fitness because offspring are more likely to inherit two identical alleles, leading to lower genome-wide heterozygosity or genetic diversity. There are two mechanisms that may explain why this is detrimental. First, overdominance at some loci means there is a specific advantage to being heterozygous. For example, individuals that are heterozygous at the regulator gene involved in cystic fibrosis are more resistant to cholera (Gabriel et al. 1994). Second, dominance at some loci can result in heterozygosity masking deleterious recessive alleles. These recessives are more likely to come together and be expressed in inbred individuals. Deleterious recessives are thought to be more important in causing inbreeding depression than overdominance (Charlesworth & Charlesworth 1999).

The detrimental effects of inbreeding have been known for a long time in captive populations. Previously, studies of inbreeding in the wild relied on data from pedigrees, which required long-term datasets and tended to be biased towards passerine birds and primates (e.g. Gibbs & Grant 1989; Dietz & Baker 1993; Keller 1998; Kruuk et al. 2002). However, the development of molecular techniques has allowed the effects of inbreeding to be examined in a greater variety of species, using estimates of heterozygosity from non-coding DNA (e.g. microsatellites). Measures of

heterozygosity, or genetic diversity, have been positively correlated with several fitness traits including juvenile survival (Coulson et al. 1998; Coltman et al. 1998; Bean et al. 2004), fecundity and reproductive success (Slate et al. 2002; Högland et al. 2002; Amos et al. 2001a), and resistance to disease (Coltman et al. 1999; Acevedo-Whitehouse et al. 2003). Further studies have also correlated genetic diversity with behavioural traits such as song diversity, aggression and competitive ability (Marshall et al. 2003; Tiira et al. 2003; Hoffman et al. 2004). These studies have indicated that even low-levels of inbreeding may be detrimental and the most genetically diverse individuals have the greatest fitness (Slate et al. 2002; Amos et al. 2001a). However, although heterozygosity of neutral markers correlates with fitness measures, the strength of these associations is usually weak (Britten 1996; Coltman & Slate 2003).

Individuals may therefore benefit from mating with maximally genetically dissimilar partners because this will increase the overall genetic diversity, or heterozygosity, of their offspring (Tregenza & Wedell 2000; Mays & Hill 2004). Polyandrous females may also select the most genetically dissimilar sperm to fertilise eggs after mating (Zeh & Zeh 1997). Disassortative mating based on the MHC suggests that these loci may be important in choosing genetically compatible mates (reviewed in Jordan & Bruford 1998; Penn & Potts 1999; Penn 2002). For example, humans prefer the smell of MHC dissimilar individuals (of the opposite sex) and mate choice in some cultures is affected by MHC dissimilarity (Wedekind et al. 1995; Ober et al. 1997; Thornhill et al. 2003).

1.2 Study species - the grey seal, *Halichoerus grypus*

1.2.1 Phylogeny

Grey seals are members of the phocid family, commonly known as the true seals, which includes the elephant seals, monk seals and many ice breeding seals. Phocids belong to the monophyletic group Pinnipedia (Árnason & Widegren 1986; Lento et al. 1995). This contains two other pinniped families: the Odobenids, the walruses, of which only one species, *Odobenus rosmarus*, is extant, and the Otariids, the eared seals, which include fur seals and sea lions. Phyletic relationships within pinniped families are generally not well resolved. There are 18 species of phocids that may be divided into two lineages or subfamilies: the monachines and the phocines (Lento et al. 1995). The monachines generally have a more southerly distribution and include the elephant seals, monk seals and Antarctic species such as the leopard seal *Hydruga leptonyx* and the

Weddell seal *Leptonychotes weddelli*. Conversely phocines tend to have a more northerly distribution and include grey seals, harbour seals *Phoca vitulina* and ice-breeding arctic species such as harp seals *Phoca groenlandica* and hooded seals *Cystophor cristata*.

There are several important features that distinguish phocids from otariids. The greatest morphological difference is in the development of the flippers. Otariids have large fore-flippers and their hind-flippers can rotate under their body so that they point forward, which allows for greater ease of movement on land. Phocids have small fore-flippers and their hind-flippers trail behind them on land. Further, most swimming action in otariids is with the fore-flippers, while phocids use their hind-flippers for propulsion through the water. The two families also differ markedly in their lactation strategies (reviewed in Le Boeuf & Campagna 1994). Phocids have a short, intense lactation period lasting from only four days in the hooded seal (Bowen et al. 1985) to six weeks in the Weddell seal (Tedman & Green 1987). They usually fast during lactation and typically remain with their pup until it is weaned abruptly. Otariids, on the other hand, have an elongated lactation period that often lasts for year, and the pup is weaned gradually. During this time the mother frequently returns to the sea to forage while the pup remains on the breeding colony.

1.2.2 Distribution

Grey seals inhabit the coastal waters of the Northern Hemisphere. There are three distinct populations: Northwest Atlantic, Northeast Atlantic and the Baltic. Divergence times for the western and eastern North Atlantic groups have been estimated at 1.0 – 1.2 million years ago (Boskovic et al. 1996). The Baltic and European populations are also genetically distinct units but diverged much later, possibly about 10 000 years ago when the Baltic Sea opened up (Boskovic et al. 1996; Graves et al. submitted).

In the Northwest Atlantic most seals occur around the Canadian Maritimes. Breeding takes place in the winter months with the two main breeding areas occurring on Sable Island, a sand bar off Nova Scotia, and on ice in the Gulf of St Lawrence. In the Baltic Sea, the grey seal population breeds mostly on ice and isolated skerries in late winter. The Northeast Atlantic population breeds in the autumn, mainly around the UK, although smaller colonies also occur on the coasts of other northern European countries.

The number of grey seals in Britain is currently estimated at approximately 109 000, 39% of the world's estimated total (Sea Mammal Research Unit, unpublished).

1.2.3 Natural history

Grey seals are sexually dimorphic; during the breeding season males may weigh in excess of 350 kg while females weigh up to 250 kg. Males may also be distinguished by their 'roman' nose and have broader necks than females. The pelage varies in colour from white to black, creating distinctive markings. Males are typically darker than females and often have few characteristic pelage markings.

Grey seals are important marine predators and as such have come into conflict with fisheries. They eat a wide variety of fish species including sand eels *Ammodytes spp.*, cod *Gadus morhua* and herring *Clupea harengus* (Murie & Lavigne 1992; Hiby et al. 1996). Diet composition varies with location, and can also change between years, which may reflect variation in prey availability (Murie & Lavigne 1992; Walton & Pomeroy 2003).

Most of the time spent in the water is at rest or feeding near specific haul-out sites (Thompson et al. 1991; McConnell et al. 1999). Foraging trips are relatively short and usually within 50 km of the haul-out site (McConnell et al. 1999; Sjöberg & Ball 2000). Nevertheless, grey seals sometimes make longer trips between haul-out sites that may be hundreds of kilometres apart (McConnell et al. 1999).

While grey seals are mostly aquatic, they aggregate annually, on land or ice, to form breeding colonies where females give birth to a single pup. Grey seals mate on the breeding colony as well, but implantation of the embryo is delayed for about three months (Boyd 1991). Grey seals also aggregate on land during the annual moult (January to March in the UK), although haul-outs are used thought out the year (Thompson et al. 1991; McConnell et al. 1992).

Grey seals are long-lived animals. Females may start breeding at three years of age but most are five before they have their first pup (Boyd 1985; Hammill & Gosselin 1995). Males reach sexual maturity slightly later at five to seven years of age (Hammill & Gosselin 1995). It is not unusual for females to live and reproduce into their 30s (Boyd

1985; Pomeroy et al. 1999) while males are thought to have shorter lifespans (Worthington Wilmer et al. 1999). Pregnancy and natality rates are high with most estimates over 90% (Boyd 1985; Hammill & Gosselin 1995; Pomeroy et al. 1999).

1.2.4 The breeding system

Although the breeding season usually spans 10 to 12 weeks at a colony, individual females have an average lactation period of only 15 to 17 days, depending on the location (Baker et al. 1995; Haller et al. 1996; Pomeroy et al. 1999). During lactation, females usually fast, relying on stored reserves to provide all the energy required for themselves and their pup. Pups are born weighing an average of 16 to 17 kg with a white lanugo that is moulted within a few weeks (Baker et al 1995; Pomeroy et al. 1999; Mellish et al. 2000). Suckling bouts last an average of 10 minutes each and occur every three or four hours (Smiseth & Lorentsen 1995; Anderson & Harwood 1985; Kovacs 1987; Haller et al. 1996). Lactating mothers lose about 4 kg of mass per day while pups gain between 1 and 2 kg (Fedak & Anderson 1982; Pomeroy et al. 1999; Mellish et al. 2000). At the time of weaning, pups weigh around 40 to 45 kg although there is substantial variation in weight both within and between years (Boyd & Campbell 1971; Bowen et al. 1992; Pomeroy et al. 1999). The pup is weaned abruptly when the mother returns to the sea and the weaned pup then remains on the colony for several weeks before going to sea in search of food.

Larger grey seal mothers generally have higher absolute maternal expenditures, longer lactation periods, and pups with faster growth rates (Anderson & Fedak 1987; Pomeroy et al. 1999). Thus, as with many pinnipeds, larger females produce larger pups at weaning (Reiter et al. 1981; Arnbom et al. 1997; Pomeroy et al. 1999). However, a relatively high expenditure one year may mean a lower expenditure in the next. As a result, some females that have had a high expenditure in one year do not breed the next year or fail to breed successfully; this is more common in young females or those with lower fat reserves (Pomeroy et al. 1999). Over their first year, larger pups have higher survival rates and female pups are more likely to survive than males (Hall et al. 2001). Although several early studies suggested mothers invest more in male offspring (Kovacs & Lavigne 1986; Anderson & Fedak 1987), more recent research has indicated that this is not the case (Bowen et al. 1992; Pomeroy et al. 1999).

Annual pre-weaning pup mortality is relatively high (North Rona 10 - 20%; Pomeroy et al. 1999) and varies substantially between colonies (Fogden 1971; Anderson et al. 1979). Starvation is the greatest cause of mortality among pups although infections, such as pneumonia and peritonitis, also occur (Anderson et al. 1979; Baker 1984). Starvation usually arises because of the permanent separation of mothers and pups (Anderson et al. 1979). The mother-pup bond forms shortly after birth, when mothers and pups smell and touch each other frequently (Fogden 1971; Burton et al. 1975). Mothers also smell the pup before suckling and may use vocal cues or location to identify the pup (Fogden 1971; McCulloch & Boness 2000). However, if the mother-pup bond fails to form, separation is likely. Fostering and milk stealing can also occur when mothers are confused about the identity of their pup (Perry et al. 1998; McCulloch et al. 1999).

Near the end of lactation, females come into oestrus and are mated (Boness & James 1979). As with all pinnipeds, male grey seals play no part in parental care. The mating system of grey seals is moderately polygynous (Anderson et al. 1979; Boness & James 1979; Amos et al. 1993; Tinker et al. 1995; Twiss et al. 1998; Worthington Wilmer et al. 1999; 2000). The primary mating tactic is the defence of a position around a group of females and thus, males do not hold strictly defined territories. Males adopting this strategy have been termed dominant or tenured males (Anderson et al. 1975; Boness & James 1979). A dominance hierarchy exists with some males consistently winning fights and thus maintaining their position within the colony (Twiss et al. 1998). Males that defend groups containing more females, and those that stay ashore longest, achieve the greatest observed mating success (Anderson et al. 1975; Twiss et al. 1994; Tinker et al. 1995; Godsell 1991; Lidgard et al. 2001). However, genetic paternity analysis has revealed that males adopting the primary tactic do not father as many pups as behavioural observations suggest they should (Amos et al. 1993; Ambs et al. 1999; Worthington Wilmer et al. 1999; 2000; Lidgard et al. 2004).

Males that do not occupy positions within the colony may adopt secondary mating tactics. These have been termed subordinate or transient males (Anderson et al. 1975; Boness & James 1979). These males gain some fertilisation success, but individually are not as successful as dominant or tenured males (Anderson et al. 1975; Twiss et al. 1994; Worthington Wilmer et al. 1999; Lidgard et al. 2004). Tactics may include

mating with females as they leave the colony, achieving 'sneaky' matings of females within or on the periphery of the colony, and aquatic mating (Boness & James 1979; Twiss et al. 1994; Ambs et al. 1999; Worthington Wilmer 1999; Lidgard et al. 2004). Males may adopt secondary strategies when they are smaller, younger and inexperienced and may subsequently adopt the primary mating tactic (Anderson & Fedak 1985; Godsell 1991; Twiss et al. 1994; Lidgard et al. 2001).

Genetic analyses of paternity in the grey seal has also revealed some evidence for female mate choice. Amos et al. (1995) reported the presence of a surprising number of full siblings, indicating mothers were faithful to the same father between years. Furthermore, those mothers that did not produce full siblings chose partners that were genetically different from previous mates, which may be a mechanism to reduce inbreeding (Worthington Wilmer et al. 2000; Amos et al. 2001b). In fact, there is evidence that lower genetic diversity, which may be caused by inbreeding, leads to lower pup survival and adult reproductive success in grey seals (Amos et al. 2001a; Bean et al. 2004).

1.2.5 Choice and effect of breeding site

Grey seals show a large amount of behavioural plasticity. The social behaviour and time budgets of grey seals differ markedly both between and within colonies due to variation in habitat (Anderson & Harwood 1985; Kovacs 1987; Caudron et al. 2001). Grey seals breed on many different types of substrate including pack ice, land-fast ice, caves, sand bars, rocky outcrops and islands. The topography can affect the numbers and density of females and pups in the colony. For instance, smaller colonies are often found in caves where conditions are crowded, especially at high tide (Anderson et al. 1979; Caudron et al. 2001). However, seals breeding on pack ice may aggregate at densities similar to land-breeding seals, despite the abundance of appropriate habitat (Lydersen & Kovacs 1999).

Grey seals are usually faithful to the colony in which they previously bred and often to which they were born (Pomeroy et al. 1994; 2000a; Twiss et al. 1994; Allen et al. 1995; Redman 2002), a feature common among pinnipeds (e.g. Baker et al. 1995; Härkönen & Harding 2001; Kretzmann et al. 2001). Philopatry may result in distant colonies becoming genetically differentiated, such as North Rona and the Isle of May which are

about 500 km apart (Allen et al. 1995). However, other colonies do not show significant geographic variation in genotypes, suggesting gene flow or recent isolation (Boskovic et al. 1996; Gaggiotti et al. 2002; Graves et al. submitted). Individuals may disperse from their natal site due to density dependant effects (Gaggiotti et al. 2002; 2004).

Within the breeding colony, females are gregarious and tend to form clusters of individuals (Anderson et al. 1975). The distribution of males is then dependent on the distribution of females. At some colonies, individual locations of both males and females are also affected by previous choice of pupping site, as they may both be highly site faithful (Pomeroy et al. 1994; in press; Twiss et al. 1994). In general though, females prefer to pup in areas with easy access to water, and these sites are often the first within a colony to be occupied (Anderson et al. 1975; Kovacs 1987; Pomeroy et al. 2000b; Twiss et al. 2000a; 2001).

In all time budget studies of grey seals, most of a female's time ashore during the breeding season was found to be spent resting, which is probably an adaptation to conserve energy (Anderson & Harwood 1985; Smiseth & Lorentsen 1995; Haller et al. 1996; Caudron et al. 2001). Comparisons within and between colonies show that the greatest difference between breeding locations is in the amount of time mothers spend in the sea (Anderson & Harwood 1985; Kovacs 1987; Caudron et al. 2001). In large colonies where females breed inland, such as North Rona and Sable Island, mothers rarely return to the sea during lactation (Anderson and Harwood 1985). This may be to prevent mother-pup separation, as females could have difficulty finding their pup upon their return. In contrast, at low densities or where females have easy access to the sea, they may spend up to 90% of their time in the water and come ashore only for nursing (Fogden 1971; Lydersen et al. 1994; Smiseth & Lorentsen 1995). While mothers often remain in shallow water close to their pups, they have also been recorded diving and thus may feed (Lydersen et al. 1994; Baker et al. 1995).

The frequency of agonistic encounters also differs between grey seal colonies as a result of the topography. When mothers return to the sea during lactation there is more movement, and hence disturbance, within the colony which can increase overall aggression rates (Anderson & Harwood 1985; Redman 2002). Density is also higher in certain habitat types, which is likely to lead to more aggression. For example, Caudron

et al. (2001) found that females pupping in small crowded caves had significantly higher rates of agonistic interactions than those pupping at lower densities.

The types of substrate that grey seals breed on can also alter their maternal behaviour. Females breeding on ice spend a greater proportion of their time in maternal behaviours than land breeders (Anderson & Harwood 1985; Haller et al. 1996). In some areas, the ice used for breeding is temporary and unstable, increasing the risk of premature mother-pup separation. This may explain why ice-breeders have a slightly shorter average lactation period of only 15 days while land-breeders usually lactate for 17 days.

Additionally, the sex ratio in the breeding colony can vary with topography (Anderson & Harwood 1985; Twiss et al. 1998; Caudron et al. 2001). At sites where access is restricted, and females are highly aggregated, the proportion of males in the colony is reduced (Twiss et al. 1998). Conversely, where colonies occur on open terrain, and females are more dispersed, many more males can gain access to the breeding colony. For example, on Sable Island (a sand bar) the sex ratio of males to females is 1:1.3 (Boness & James, 1979) while on North Rona (access is restricted by several gullies leading to the sea) the sex ratio is about 1:7 (Anderson et al. 1975). Unsurprisingly, at sites with a higher ratio of males to females, agonistic encounters between males are more frequent (Anderson & Harwood 1985; Twiss et al. 1998; Caudron et al. 2001). However, the degree of observed polygyny at all sites was found to be similar, regardless of the sex ratio (Twiss et al. 1998).

1.3 Study site - North Rona

North Rona (59° 06´ N, 05° 50´ W) forms part of the Outer Hebrides and lies 75 km N.N.W. of Cape Wrath, Scotland (Figure 1.1). The island is approximately 1.2 km^2 with the highest point at 108 m above sea level (Figure 1.2). The most recent colonisation of North Rona by grey seals is thought to be around 1844 when the last human inhabitants left the island (Boyd et al. 1962). In the late 1800s seals were being harvested from North Rona but in 1956 it was declared a National Nature Reserve along with the neighbouring island of Sula Sgeir (Boyd et al. 1962). Thereafter, a considerable number of studies were carried out to gain a better understanding of the grey seal colony. The first of these mainly aimed to described the colony and estimate pup production in order to determine how best to manage the population (Boyd et al. 1962;

Boyd & Laws 1962; Boyd & Campbell 1971; Summers et al. 1975). More recent studies have focussed on physiology, social behaviour and genetics (Anderson et al. 1975; Fedak & Anderson 1982; Anderson & Fedak 1987; Amos et al. 1993; 1995; 2001a,b; Pomeroy et al. 1994; 1999; 2000a; 2001; Twiss et al. 1994; 1998; 2002; 2003; Worthington Wilmer et al. 1999; 2000; Redman et al. 2001).

Figure 1.1: The location of North Rona, indicated by the black star, on a map of Scotland.

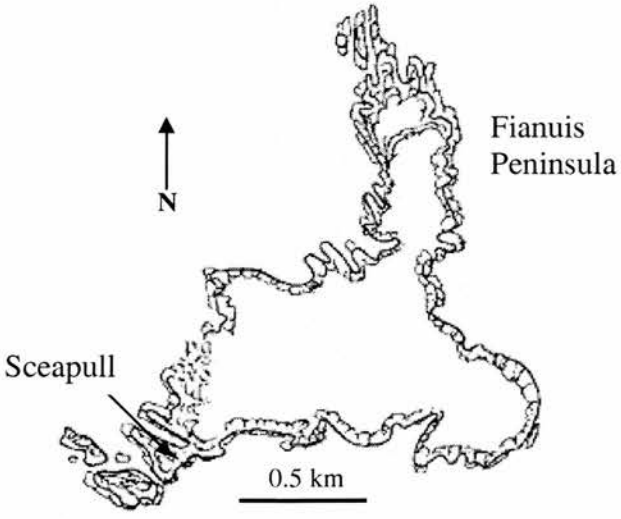


Figure 1.2: Map of North Rona showing the two areas used for breeding.

Studies in the 1960s reported an average of about 2,300 pups born annually on North Rona (Boyd & Laws 1962; Boyd & Campbell 1971). At that time it was the largest

breeding colony in the UK (Boyd et al. 1962). However, the number of pups born on North Rona declined in the 1970s and 1980s. Currently, the population size is relatively stable, with an annual pup production of around 1000 (C. Duck pers. comm.).

Most pup production on North Rona occurs on the Fianuis peninsula (95%), with the remainder of pups born on Sceapull (Figure 1.2; Boyd & Laws 1962; Boyd et al. 1962). The peninsula is mostly cliff-bound and seals come ashore via gullies that lead to the sea. The Fianuis peninsula is characterised by low-lying undulating terrain. As with the rest of the island, it is grass-covered and quite uneven with many boulders and rocky outcrops. A large number of irregularly spaced small pools are available throughout. These tend to be more prevalent later in the breeding season, when the ground becomes saturated with water.

Grey seals use the island for breeding from late September to late November, with pup production reaching a peak in early to mid October (Summers et al. 1975; Hiby et al. 1996). During the remainder of the year, seals use the island as a haul-out location. Temperatures during the breeding season average around 10 °C but range between 3 °C and 14 °C (Fedak & Anderson 1982; Redman et al. 2001; Twiss et al. 2002). Typically, conditions at this time are also fairly wet with frequent rainfall (Fedak & Anderson 1982; Redman et al. 2001; Twiss et al. 2002).

Previous studies of the grey seal colony on North Rona provide important resources and background information that enable detailed questions to be asked about the colony. Firstly, many seals on the colony are individually recognisable. Seals have been branded and tagged since the 1960s (Boyd & Laws 1962; Boyd & Campbell 1971). The last animals were branded in the mid 1990s (some of which are still breeding) while tagging continues to the present day (Pomeroy et al. 1999). Currently, the less intrusive method of photo-identification, which uses unique pelage markings to distinguish individuals, is being used (Redman 2002). On North Rona, a pelage database of females was created in 1998 and is updated every field season (Redman 2002). The 2003 database holds 266 females that can be recognised between years from photographs (S. Ruddell pers. comm.). Males can also be identified between years using pelage markings, although a photographic database is not available. This reduces the amount of genetic sampling, as adults only need to be sampled once in their lifetime while information about individuals may be collected over numerous years.

Secondly, fine-scale habitat characteristics of the main area of study have been incorporated into a geographic information system (GIS) database (Twiss et al. 2000a,b; 2001; 2002; 2003). This can be used to extract fine-scale information about the physical and social characteristics of a location (as in Pomeroy et al. 2001; Twiss et al. 2002; 2003). Differences in habitat quality of pupping sites, which vary markedly across the colony, may then be taken into account in analyses. The GIS also provides access to high-resolution maps (derived from aerial photographs) of parts of the colony to allow locations of individuals to be accurately recorded in the field.

Previous studies have also identified several features of the North Rona colony that suggest some form of sociality may be present. On North Rona, mothers remain with their pups throughout lactation and thus also remain close to neighbouring females (Anderson et al. 1975). On other colonies, mothers often leave their pups to go to water and consequently interact with many different females (Fogden 1971; Anderson & Harwood 1985; Redman 2002). Females on North Rona are also highly site faithful to their pupping sites and may therefore interact with the same individuals over many years (Pomeroy et al. 1994; in press). However, females do not exhibit site fidelity in other colonies, such as Sable Island, where there is little variation in habitat (Boness & James 1979). Passive inter-annual associations would be unlikely to form in these other colonies. Recent work also suggests the presence of active associations between females on North Rona, suggesting the existence of some form of long term sociality (Pomeroy et al. in press). Other research has indicated that the colony is genetically structured, with those breeding in good habitat having more relatives in the colony (Pomeroy et al. 2001). The presence of a basic form of both sociality and genetic structuring make this an interesting system to investigate patterns of kinship and genetic diversity and their effect on behaviour.

Chapter 2: Characterisation of microsatellite loci and a comparison of relatedness estimators

2.1 Introduction

Microsatellites are frequently used in studies of kin selection, population genetic structure and quantitative genetics. They are composed of motifs of up to six base pairs that are tandemly repeated. Microsatellites have several characteristics that make them useful to researchers. Firstly, they are essentially ubiquitous and have been found in all organisms studied thus far, from bacteria to vertebrates (Hancock 1999). Most microsatellite loci are also under neutral selection and thus mutation rates should be constant through time and across populations (Schlötterer 2000). Finally, microsatellites have a high mutation rate, thought to be 10^{-4} to 10^{-3} mutations per locus per generation in mammals (Zhivotovsky et al. 2000; Whittaker et al. 2003). This results in the variation necessary for detecting differences between individuals and populations.

Most microsatellite mutations are thought to occur during replication when the two strands of DNA become misaligned. This can lead to an increase or decrease in the number of repeat motifs and is known as replication slippage (Levinson & Gutman 1987). The stepwise model of mutation, which assumes each mutational event involves the loss or gain of one repeat unit, has classically been proposed to describe this mutational process (Kimura & Ohta 1978). In this model alleles may mutate back to previous states already present in the population. This is in contrast to the infinite allele model that proposes that a mutational event always creates a new allelic state (Kimura & Crow 1964). The infinite allele model is sometimes seen as a poorer description of microsatellite mutations than the stepwise model, especially when examining events further back in a pedigree. However, the stepwise model probably remains an oversimplification of the microsatellite mutational process as many factors may influence mutation rate (Ellegren 2000). For instance, mutation rate increases exponentially with microsatellite length and as length increases mutations that shorten the microsatellite may become more likely (Whittaker et al. 2003).

Microsatellites may be used to estimate genetic relatedness between pairs of individuals. This is particularly useful for wild populations, when pedigrees are not available, and to minimise inbreeding in captive-breeding programmes. In recent years, several pairwise relatedness estimators have been constructed for use with microsatellite

loci (Queller & Goodnight 1989; Lynch & Ritland 1999; Wang 2002). These use the amount of allele sharing between two individuals and the frequencies of those alleles in the population to estimate the relatedness between individuals. Two individuals may share the same allele because of identity by descent, if they share a recent common ancestor. However, two alleles may be the same because they arose more than once through mutation and are thus identical by state only. In addition, "alleles that are identical by state might not be identical by descent if they coalesce further back than the reference pedigree" (Blouin 2003). Individuals are more likely to share rare alleles through identity by descent. Hence, pairs that share rare alleles have higher relatedness values than those that share common alleles, which may be due to identity by state only. Therefore, relatedness may be defined as a measure of the fraction of alleles shared among individuals that are identical by descent (Blouin 2003). Unrelated individuals are expected to have a relatedness value of zero and values can be negative if a pair is less related than the population average. The relatedness between first-degree relatives (parent-offspring and full siblings) is expected to be 0.5 as they share an average of half of their alleles. Similarly, second-degree relatives (half siblings, grandparents-grandoffspring, aunts and uncles–nieces and nephews) share an average of a quarter of their alleles and are expected to have a relatedness value of 0.25.

Sampling variance of relatedness estimates is always high because estimates of identity by descent vary among loci and also because of the possibility of sharing alleles that are identical by state only (Lynch & Ritland 1999; Blouin 2003). However, there are several possible ways to lower variance. One is to increase the number and variability of loci, but this is not always practical (Lynch & Ritland 1999). Another is to choose an appropriate relatedness estimator for a dataset. The performance of an estimator will depend on the number and frequency distribution of alleles at each locus (Lynch & Ritland 1999) and the population composition (i.e. the proportion of close relatives; Van de Casteele et al. 2001). The sampling variance for each estimator will also depend on the type of relationship being tested (e.g. mother-offspring). Therefore, different estimators should be tested with several types of relationships to determine which yields the lowest variances for a population (Van de Casteele et al. 2001).

Three of the most commonly used relatedness estimators are described below:

1) *Queller & Goodnight (1989)* (denoted as QG). This estimator was originally designed to compare the average relatedness between groups of individuals but can also be used to estimate pairwise relatedness. Asymmetric estimates based on individual *x*'s relationship to individual *y* and individual *y*'s relationship to individual *x* are calculated. Asymmetric estimates will differ because the frequency of the shared alleles may differ between individuals (i.e. *x* may be a homozygote and have two copies of the shared allele and *y* may be a heterozygote and have only one copy). Also, estimates are calculated using the population frequencies of the alleles of the reference individual (either *x* or *y*) only. The mean of the asymmetric estimates is taken to give a symmetrical pairwise estimate. For multilocus estimates, all loci are weighted equally.

2) *Wang 2002* (denoted as WG). This is a modification of the Li et al. (1993) similarity index and has been developed to behave well when population allele frequencies have sampling errors due to the inclusion of relatives and small sample sizes. For multilocus estimates, loci are weighted by the inverse of the amount of information provided by each locus. This value is based on the allele frequencies at each locus.

3) *Lynch & Ritland (1999)* (denoted as LR). This estimator uses a regression approach. A pair's relatedness is defined as twice the probability that, for any locus, a random gene taken from an individual *x* is identical by descent with a random gene taken from individual *y*. For multilocus estimates, loci are weighted by the inverse of the sampling variance for the locus-specific estimates. Sampling variance will be higher for loci that provide less information (i.e. less variable loci with few alleles) and therefore these will contribute less to the overall estimate.

The performance of these relatedness estimators was tested on three datasets of grey seals (i.e. mother-offspring, half siblings and unrelated) sampled from the North Rona breeding colony. Microsatellites were used to estimate pairwise relatedness, as extensive pedigrees do not exist for the colony. These estimates were subsequently used to investigate spatial and temporal patterns of relatedness of breeding females (Chapter 3 and 4) and increase understanding of grey seal behaviour (Chapter 5 and 6).

Genotypes were also used to obtain individual genetic diversity estimates (Chapter 4 and 5) and assign parentage (Chapter 6). Microsatellites have previously been used in studies of grey seals to estimate relatedness (Pomeroy et al. 2001; Redman 2002) and genetic diversity (Amos et al. 2001a; Bean et al. 2004) and to investigate patterns of parentage (Ambs et al. 1999; Worthington Wilmer et al. 1999; 2000; Lidgard et al. 2004) and genetic differentiation of colonies (Allen et al. 1995).

2.2 Aims

The aim of this chapter was to provide a description of the microsatellite loci used in this thesis and demonstrate that they possess the properties necessary to provide robust estimates of relatedness and genetic diversity. The genotyping error rate was estimated to ensure consistency in scoring alleles both within and between laboratories. This involved comparing the genetic results to those from a complementary pelage identification study. A second objective of this chapter was to identify the most appropriate relatedness estimator for this population by comparing performances on three different datasets. Once this was completed a suitable cut off point was chosen to enable pairs to be classified as related or unrelated.

2.3 Methods

2.3.1 Genetic samples

This thesis used skin samples collected from grey seals in the North Rona breeding colony from 1997 to 2003 (Table 2.1). All procedures on live animals were carried out under Home Office Licence. When samples were taken from adults, photos, video images, or sketches were collected when possible. These have been used successfully as a non-intrusive way to identify individuals returning to the colony in following years (Redman 2002). Some adults also had individual brands on their flank or tags between the digits of their hind flippers, which were applied for other studies (see Pomeroy et al. 1999 for details). Many adults that could be individually identified were observed in several seasons but were only sampled once.

Table 2.1: Numbers of grey seals sampled (S) and genotyped (G) at more than six loci each year. From 1997 to 1999 only the sub sample of individuals that was selected to be genotyped was listed under sampled. More samples were collected in these years but were not used in this thesis.

Year	Females		Males		Live Pups		Dead Pups	
	S	G	S	G	S	G	S	G
1997	160	142	0	0	0	0	0	0
1998	56	55	0	0	0	0	0	0
1999	5	5	0	0	0	0	0	0
2000	62	62	17	17	40	40	2	1
2001	135	130	27	22	81	80	13	6
2002	94	91	23	23	53	52	18	13
2003	14	13	22	19	30	29	7	5
Total	**526**	**498**	**89**	**81**	**204**	**201**	**40**	**25**

From 2000 to 2002 samples were collected mainly from the Study Area for four purposes: 1) All adults involved in behavioural observations were sampled when possible to obtain pairwise relatedness and genetic diversity estimates (Chapters 5 and 6). 2) Mothers and pups were captured throughout the colony as part of a long-term study of reproductive performance. Skin samples were taken to obtain genetic diversity estimates, which may help explain differences in individual quality (these were used as part of another study) and for paternity analysis (Chapter 6). 3) When matings were observed, females, males and pups in the subsequent year were targeted to determine whether the male had been successful in siring the pup (Chapter 6). 4) Samples were also taken opportunistically from males and females in the Study Area to increase the overall sample size and obtain individuals from areas not previously sampled. These were combined with other samples to investigate patterns throughout the Study Area (Chapter 3, 4 and 6). The purposes outlined above were not mutually exclusive and a sample often fell into more than one category.

This thesis also includes individuals sampled from 1997 to 1999 and 2003 (Table 2.1). In 1997, samples were collected from the three main pupping regions in the colony, the Study Area, Fianuis South and Fianuis North, giving samples from a much wider geographic range than in other years. Some samples from 1997 (n = 96) were genotyped by A. Overall in W. Amos's lab at the University of Cambridge and these genotypes were used only in Chapters 3 and 4. Many of the 1998 and 1999 samples had also previously been genotyped in Cambridge but these genotypes were not used in this thesis. However, a sub-sample (n = 50) of the 1998 samples was genotyped as part of a

compatibility study between the two labs (see below). The few other samples collected in 1998 and 1999 that were genotyped for this thesis (Table 2.1) were specific individuals that were resighted on the colony in 2001 or 2002. In 2003, only specific individuals were targeted for sampling. Generally, these individuals formed part of the 2001 and 2002 behavioural observations but were not sampled in those years. In addition, mothers and pups that were part of reproductive performance studies were also sampled, as well as any unsampled males from the Study Area. Data from samples collected in 2003 were only used in Chapters 5 and 6 as they were processed after the completion of other analyses.

2.3.2 Microsatellite genotyping

A small skin sample (~2-5 mm^2) was taken from individuals. For all pups and for females that were part of the reproductive performance study, skin was taken from the trailing edge of a hind flipper (females) or the tail (pups) using ear-notching pliers. Otherwise, females were sampled using a biopsy head attached to a pole. Males were skin sampled using the remote biopsy technique described in Gemmell & Majluf (1997). Samples were stored in either 20% DMSO or 96% ethanol (2002 and 2003) and frozen at –20 °C. DNA was extracted from a small piece of tissue (~ 1 mm^2) by placing it in 700 µl of lysis buffer (0.15 M NaCl, 50 mM Tris, pH 8.0, 1.0 mM EDTA) and adding 15 µl of proteinase K solution (20 mg/µl) and 40 µl of 20% SDS. This was incubated at 45 °C overnight followed by standard phenol/chloroform extraction (Sambrook et al. 1989). The DNA was precipitated using 3 M sodium acetate, pH 5.2 and 100% isopropanol and cooling at -70 °C for one hour. The DNA was washed with 70% ethanol before being resuspended in TE (10 mM Tris, pH 7.5, 1 mM EDTA). DNA was run on agarose gels with standards to estimate the concentration of DNA in each sample. Samples were diluted to a concentration of 10 – 20 ng/µl.

Samples were amplified at 11 microsatellite loci (Table 2.2). Of these, nine (not Hl15 and Lc6) had previously been used with grey seal samples from North Rona (Allen et al. 1995; Worthington Wilmer 1999; 2000). Four other loci were tested for their usefulness to this study but were not used (Table 2.2). Primer sequences were taken from the references listed in Table 2.2 except for Hgdii for which a new reverse primer was designed (AGG ACT CCT GCC ACT GAG AA). PCR reactions were performed in 0.2 µl tubes for polyacrylamide gel electrophoresis (PAGE) and 96 well microtitre

plates for the automatic system (see below). Each reaction contained 1 µl of DNA, 1 µl Promega 10x buffer, 1.25-1.75 mM of $MgCl_2$ (Table 2.2), 2 pmol of each primer, 0.05 mM each of dATP, dCTP, dGTP and dTTP and 0.375 units of Taq polymerase (Bioline), made up to 10 µl with sterile distilled water. Reactions for three loci (Hl16, Hl20 and Lw11) also contained 60 mM of tetramethylammonium chloride. Reactions were overlaid with mineral oil and amplifications performed in a PTC-100 Programmable Thermal Controller, MJ Research, Inc. A 2 min denaturation step at 94 °C was followed by 30 cycles of 10 seconds of denaturation at 94 °C, 30 seconds of annealing at the temperature indicated in Table 2.2, and 30 seconds of extension at 72 °C and ending with 5 minutes at 72 °C. Five additional cycles were added to the programme for Hl15 and Lc6 to increase yield.

Table 2.2: Source and PCR specifications for microsatellite loci.

Locus	Reference	Annealing temp. (°C)	$MgCl_2$ (mM)	Fluorescent dye
Loci used in this thesis				
Hg3.6	Allen et al. 1995	58	1.5	D4
Hg4.2	Allen et al. 1995	58	1.5	D3
Hg6.1	Allen et al. 1995	62	1.5	D3
Hg6.3	Allen et al. 1995	60	1.75	D4
Hg8.9	Allen et al. 1995	58	1.5	D4
Hg8.10	Allen et al. 1995	60	1.25	D2/D3
Hgdii	Allen et al. 1995	56	1.25	D3
Pv9	Goodman 1997	52	1.5	D4
Pv11	Goodman 1997	60	1.25	D4
Hl15	Davis et al. 2002	56	1.25	D3
Lc6	Davis et al. 2002	55	1.25	D4
Loci tested but not used				
Hl16	Davis et al. 2002	56	1.25	
Hl20	Davis et al. 2002	52	1.25	
Lc26	Davis et al. 2002	66	1.25	
Lw11	Davis et al. 2002	56	1.25	

At least ten samples for each locus were run on a denaturing 6% polyacrylamide / 6 M urea gel. An equal volume of formamide dye was added to each PCR product, which was then denatured for 3 min at 95 °C before 5 µl was loaded into the gel. A 10 bp ladder (Invitrogen) was run to size alleles. Alleles were visualised by silver staining following Promega's protocol and applications guide. Samples were run using PAGE so that genotypes could be directly compared to those from the automated system. This was necessary in order to combine results from this lab with those from W. Amos's lab in Cambridge where nine loci had previously been genotyped using PAGE. In addition,

for loci that had not previously been used with grey seals, PAGE was a useful way to test for the degree of polymorphism and aid in optimising conditions.

For the 11 loci chosen for this study, PCR products were run on a Beckman Coulter CEQ system using the Frag 3 programme. One primer from each pair was labelled with either D3 (green) or D4 (blue) fluorescent dye (Invitrogen) (Table 2.2). Hg8.10 was originally labelled as D2 (black) but this colour gave a considerably weaker signal than the others and proved unusable. Between 0.5 and 1.0 µl of each PCR product was added to 40 µl of ultra pure formamide and 1.2 µl of CEQ DNA size standard – 400 (Beckman Coulter) in a 96-well plate and run following Beckman Coulter CEQ protocols. Three to four PCR products from different loci were run together with neighbouring loci labelled with different colours and overlap between alleles minimised. The 2000 and 2001 samples (except for Hg8.10, Pv11, Hl15, and Lc6) were run and analysed using CEQ 2000XL software. Alleles were identified by eye from computer-generated traces with peak sizes labelled. Genotypes were then manually entered into an EXCEL spreadsheet. For all other samples, CEQ 8000XL software was used. Computer generated fragment traces were again visualised but peaks representing alleles were automatically identified and placed into 'bins' and assigned identities. Bin width was set at 0.8 bp to allow for differences between samples and runs. All results were then checked by eye and automatically transferred to an EXCEL spreadsheet.

2.3.3 Number of loci used

This project began by using the first eight microsatellite loci listed in Table 2.2. However, due to the use of black fluorescent dye, Hg8.10 was unreadable the first time it was run. In order to investigate the ability of the remaining seven loci to accurately estimate relatedness, simulations were performed on Kinship 1.0 (Queller & Goodnight 1999). Kinship was used for these simulations solely because it was the most commonly used and easily available programme at the time. Datasets of 1000 pairs were created using three types of relationships: parent-offspring (PO), half siblings (HS) and unrelated (UR). If the seven loci were not very powerful, large variances would be associated with the estimates and little confidence could be placed in them. These simulations used allele frequencies from the 1999 to 2002 adults (n = 282) as those from 1997 and 1998 had not been completed at the time. The results from these simulations suggested that more loci needed to be added (see results) and so the

remaining three loci in Table 2.2 plus Hg8.10 were genotyped. The simulations were then repeated using all 11 loci and allele frequencies from the 1997 to 2002 adults (n = 412).

2.3.4 Identity checking

Although every effort was made not to resample the same animal, this inevitably occurred because individuals were not recognised in the field. There were two types of duplicate genetic samples i) those that were matched first using pelage and ii) those that were matched first using genotypes. Samples that were matched first using pelage were used to estimate the rate of genotyping errors (see below). All other samples were checked for genetic matches using the Identity function in CERVUS 2.0 (Marshall et al. 1998) so that the final dataset included each individual only once. All adults genotyped for at least seven of the same loci were compared allowing for up to two mismatches. The probability of an identical genotype occurring by chance in two different individuals at the seven least polymorphic loci was calculated following Paetkau & Stroebeck (1994). When mismatches were found between samples, microsatellite traces were compared to identify binning errors. If this did not locate the inconsistency, both samples were rerun. Mother-pup pairs were also tested for mismatches using CERVUS 2.0 (Marshall et al. 1998). When a pair did not share at least one allele at each locus, microsatellite traces were checked first before rerunning both the mother and the pup.

Each sample was originally given a unique identity. When photographs (females) or sketches (males) corresponding to both samples from a genetic match were available, these were compared. The purpose of this comparison was to confirm that, for these individuals, no genetic samples or photos had been mislabelled. Comparisons of pelage markings of photographed females were performed independently by S. Ruddell. Photos were matched by eye using a number of identifying features, including the position of the counter-shading line and the size, shape and position of spot pattern components (see Redman 2002 for more details). S. Twiss independently compared pelage markings of males from field sketches.

2.3.5 Error rates

Some samples were retrospectively found to be same using pelage markings before the genetic comparison was made. Female matches were made in the field using

photographs or sketches by P. Pomeroy, P. Redman, S. Ruddell, or myself. All matches were confirmed using two observers. Retrospectively, females were matched using photographs by P. Redman or S. Ruddell. S. Twiss matched all the male samples. These samples were used to estimate genotyping error rate. Although I often knew the identity of duplicate samples, they were effectively run blind as samples were renamed for genotyping and the real identities not assigned until the comparison was made. Separate error rates for each locus were taken as the percentage of mismatched genotypes at that locus. The overall error rate per locus was estimated as the total number of mismatches divided by the total number of genotyped loci for all individuals times one hundred. This takes into account those individuals in the comparison that were not genotyped for all 11 loci.

An error rate was also estimated between the lab at St. Andrews and W. Amos's lab at the University of Cambridge to ensure there were no systematic differences in genotyping. Fifty female samples from 1998 that had previously been genotyped in Cambridge were re extracted from tissue and genotyped. Genotypes were then sent to the Cambridge lab where W. Amos made the comparison. Since samples at the Cambridge lab had not been genotyped for Hl15 and Lc6 these were not included in the analysis. An overall error rate per locus was estimated as above.

2.3.6 Population statistics

A dataset containing all adults from 1997 to 2002 with no duplicate samples (n = 412) was used to check for deviations from Hardy-Weinberg equilibrium, using exact tests, and for linkage disequilibrium in Genepop 3.1c (Raymond & Rousset 1995). Both of these tests used a Markov chain algorithm with 1000 dememorization steps, 100 batches and 1000 iterations. The observed heterozygosity and the frequency of null alleles at each locus were calculated using CERVUS 2.0 (Marshall et al. 1998). The frequency of null alleles was estimated using an iterative algorithm based on the difference between the observed and expected frequency of homozygotes.

2.3.7 Relatedness estimators

Datasets were constructed from genotyped individuals to represent three types of relationships. First, 125 mother-pup (parent-offspring, PO) pairs were selected so that each mother was used only once. Second 41 pairs of maternal half-siblings (HS) were

selected. There were several mothers that had pups sampled in three years and in these cases all pups were used to make all possible comparisons to increase the sample size. Parentage analysis was performed using CERVUS 2.0 to test for possible full siblings (Marshall et al. 1998). The number of candidate males was set to 450. CERVUS 2.0 also required a genotyping error rate, which was estimated at 1% (see results) and the proportion of typed loci, which was set to 99%. Half siblings were excluded when they had at least an 80% chance of being assigned the correct father and that father was the same. However, this is unlikely to pick up all full siblings, as those with an unsampled father would not be excluded. Finally, a random set of 125 female-female pairs was chosen using the random number generator in EXCEL; these were assumed to be unrelated pairs (UR). Using background allele frequencies from 412 adults, relatedness values were calculated for the three estimators (QG, WG, LR) using the programmes listed in Table 2.3. All estimates were tested for deviation from their expected value (UR = 0, HS = 0.25, PO = 0.5) and the correlation between relatedness values for different estimators was compared. Statistical analyses were performed in SPSS 11.0.

Table 2.3: Programmes used to estimate relatedness.

	Programme	Reference	Website
QG	RELATEDNESS 5.0	Queller & Goodnight 1989	http://www.gsoftnet.us/GSoft.html
WG	MER	Wang 2002	http://www.zoo.cam.ac.uk/ioz/software.htm
LR	DELRIOUS	Stone & Björklund 2001	http://www.science.mcmaster.ca/biology/faculty/stonej/SOFTWARE/DELRIOUS/delrious.htm

2.4 Results

A total of 805 samples were genotyped for at least seven loci (Table 2.1). There were 54 samples collected that failed to amplify. This was largely due to small tissue samples not yielding sufficient DNA but in some cases, particularly with dead pup samples, DNA was degraded. There were also a relatively large number of failed samples from 1997, which may have been due to the age of the tissue (Table 2.1). For genotyped samples, 90% (727/805) were completed for all 11 loci. All but 13 of the remaining samples were genotyped at 10 loci. Fewer than five samples were not genotyped for most loci while Lc6 was not genotyped for 68 samples (Table 2.4).

Table 2.4: Number of alleles, observed heterozygosity, and estimated frequency of null alleles for 11 microsatellite loci calculated for adult samples (n = 412). Shift refers to the increase in the number of base pairs observed using the Beckman-Coulter system compared to those run on polyacrylamide gels.

Locus	Number Genotyped	Number of Alleles	Observed Heterozygosity	Estimated Frequency of Null Alleles	Shift
Hg3.6	800	8	0.791	-0.006	0
Hg4.2	801	10	0.672	-0.040	2
Hg6.1	801	6	0.600	0.014	0
Hg6.3	801	6	0.780	0.001	4
Hg8.9	798	11	0.864	-0.018	2
Hg8.10	797	10	0.778	0.009	-2
Hgdii	804	8	0.687	-0.005	-2
Pv9	800	7	0.777	0.019	-2
Pv11	802	8	0.677	0.023	-2
Hl15	802	17	0.871	-0.004	4 to 6
Lc6	737	11	0.774	0.022	0
Mean	795	9.3	0.755		

2.4.1 Microsatellite characterisation

The number of alleles at each locus varied between 6 and 11 with the average heterozygosity being 0.755 (Table 2.4). Allele frequencies from 412 individual adults are listed in Appendix 2.1. The frequency of null alleles was low for all loci (Table 2.4). All loci were in Hardy-Weinberg equilibrium with $p > 0.05$ with the exception of Hg6.3 ($p = 0.048$). This was not significantly out of Hardy-Weinberg equilibrium after Bonferroni corrections for multiple comparisons. Analysis of linkage disequilibrium found two pairs of loci (Hg6.1 & Hg8.9 and Hg8.10 & Lc6), out of a possible 55, had $p < 0.05$. Again, these were not significant after Bonferroni corrections for multiple comparisons. When allele sizes were compared between samples run on the Beckman Coulter CEQ system and PAGE, allele sizes were found to shift between 0-6 bp (Table 2.4). For Hgdii, where a new primer was designed, individuals run with the old and new primer were compared. From the allele size using the new primer on the Beckman Coulter CEQ system, 74 bp were deducted for it to be equivalent to the old primer on PAGE. A new locus, Hl15, did not appear to correlate exactly between PAGE and the Beckman Coulter CEQ system. Specifically, the two smaller alleles (105 and 113) were 6 bp shorter on the Beckman Coulter CEQ system while the remaining alleles were only 4 bp shorter. However, this locus was consistent with allele size on the Beckman Coulter CEQ system. In future, if results from PAGE are available for Hl15, care must be taken before combining genotypes taken from the two methods.

Four loci were tested that were not subsequently used in this study for reasons outlined in Table 2.5. Hl20 was originally selected for use on the Beckman Coulter CEQ system. However, after approximately 100 samples had been completed, it was apparent that allelic dropout or null alleles were a serious problem as a very high proportion of individuals were homozygotes. Therefore, this locus was dropped from the study. Two loci were not included because they were difficult to interpret (Table 2.5). These loci were consistently associated with a large amount of smearing and stuttering making it difficult to identify the bands that corresponded to alleles. The final locus (Lw11) had only three alleles, one of which was present in all samples. Thus, this locus would have provided little additional information.

Table 2.5: Characteristics of loci tested but not used in this thesis.

Locus	Number Genotyped	Number of Alleles	Size range	Reason for not using
Hl16	18	4?	135-141	Difficult to interpret
Hl20	22	5	110-118	High allele dropout
Lc26	11	5	~ 310	Difficult to interpret
Lw11	10	3	172-176	Too few alleles

2.4.2 Number of loci used

Simulations using allele frequencies from seven loci showed a high degree of overlap between the three datasets representing different relatedness categories (Figure 2.1A). When the number of loci was increased to 11, overlap between datasets decreased (Figure 2.1B). Variances decreased for the HS and UR datasets from approximately 0.040 to 0.025 while those from the PO dataset dropped from 0.018 to 0.012, for datasets using seven and 11 loci respectively. All datasets were normally distributed (Kolmogorov-Smirnov goodness-of-fit test, $p > 0.05$). Relatedness estimates for simulated datasets were calculated using the Queller and Goodnight (1989) relatedness estimator.

2.4.3 Identity checking

Within the 1997 to 2002 dataset a total of 39 pairs, 11 sets of three, and two sets of four duplicated samples were matched first using genotypes. Only one of these pairs was male. Thus 14% of females and 2% of males were resampled individuals that were identified first though genetic matching. The likelihood of two individuals having

identical genotypes for the seven least polymorphic loci was 2.0 x 10^{-7}, suggesting identical genotypes were the result of resampling. All but one matched pair was genotyped at more than seven of the same loci. All mismatches between pairs were corrected after rechecking fragment traces or rerunning samples.

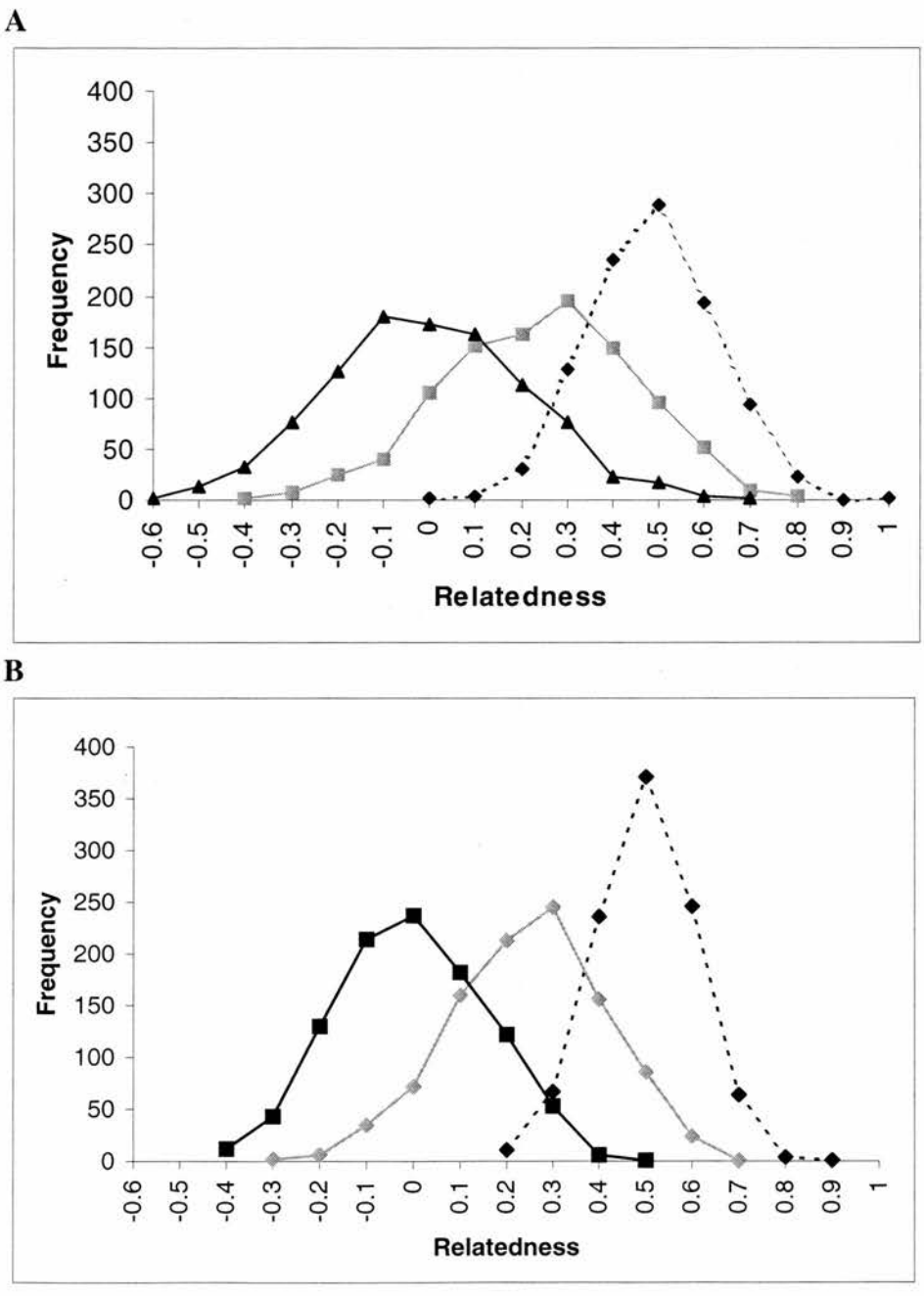


Figure 2.1: Distribution of relatedness estimates from simulated parent-offspring (dashed black line), half sibling (solid grey line) and unrelated (solid black line) datasets using allele frequencies from A) seven loci and B) 11 loci.

Of the resampled individuals only 18 females pairs and the male pair had photographs (females) or sketches (males) that could be compared. When these were examined, all agreed with the genetic matches. Pelages of remaining genetic matches could not be compared because samples did not have corresponding photographs or the quality of the photographs was too low.

The genotypes of mothers were compared to those of their pups to ensure they shared one allele at each locus. Of a possible 168 mother-pup pairs, 16 were found to have mismatches at one locus and these were all corrected after rechecking fragment traces or rerunning samples. Two samples had more than one mismatch. In both cases, when the mother and pup were rerun genotypes were recorded as the same as the original genotypes, indicating that they were not a mother-pup pair.

2.4.4 Error rates

For the 127 samples that were involved in known matches from pelage (45 female pairs and four sets of three, and eight male pairs and three sets of three), there were 14 genotyping errors. Mismatches between samples never occurred at more than one locus and the incorrect genotype at a locus was identified and corrected in all cases. Error rates varied between loci with Pv9 and Hl15 having the highest rates while no errors were found in three loci (Table 2.6). The overall error rate per locus was 1.0%.

Table 2.6: Error rates per locus with possible explanations for errors. See text for a description of the error types.

Locus	Number of errors	Error rate (%)	Possible reasons for errors			
			Binning	Tube Mislabelling	Allelic dropout	Unknown
Hg3.6	2	1.6	1			1
Hg4.2	1	0.8	1			
Hg6.1	1	0.8			1	
Hg6.3	1	0.8				1
Hg8.9	1	0.8				1
Hg8.10	0	0				
Hgdii	0	0				
Pv9	4	3.1		2		2
Pv11	1	0.8				1
Hl15	3	2.4	1		1	1
Lc6	0	0				
Total	14		3	2	2	7

The cause of errors was only apparent in some cases (Table 2.6). A binning error occurred when the fragment trace was correct but the data was miscopied or an allele incorrectly assigned. Tube mislabelling is often difficult to detect and was identified in only one case when samples were run side by side for the Pv9 locus. After errors in these samples were identified at this locus, examination of the genotypes suggested that the sample labels had been swapped during either amplification or genotyping. Allelic dropout was recorded when the incorrect genotype was a homozygote for one of the alleles in the correct genotype. While allelic dropout may have been responsible for some errors, this does not appear to be a consistent problem in any locus.

Of the errors listed in Table 2.6, six involved comparisons between genotypes in which one was typed using the automatic binning system, and then checked by eye (CEQ 8000XL software), and one was typed only by eye (CEQ 2000XL software). In five cases, the correct genotype was identified using the automatic binning system first.

Of the 50 samples chosen for the comparison with the Cambridge lab one failed to amplify, leaving 49 samples. When these were compared to genotypes from the Cambridge lab five errors were identified giving an error rate of 0.9%. Two errors occurred in Hg 6.1 and one error occurred in each of Hg3.6, Hg8.10 and Hgdii. Additionally, there were 10 matches between the 96 samples from 1997 that were genotyped in the Cambridge lab (used in Chapters 3 and 4), and genotypes from samples collected from 1998 to 2002. Although error rates were slightly higher than expected (4 errors) they also showed no systematic differences between labs.

2.4.5 Relatedness estimators

All datasets for all estimators were normally distributed (Kolmogorov-Smirnov goodness-of-fit test, $p > 0.05$). Only the LR estimate for UR dataset deviated significantly from the expected value ($r = 0$) (Table 2.7), although this difference was not significant after Bonferroni corrections for multiple comparisons (i.e. at $p < 0.006$). When this dataset was removed, each relatedness estimator had the lowest variance in one category (Table 2.7). The variances of the relatedness estimates differed significantly between the three relatedness estimators for the UR datasets (Bartlett's test for homogeneity of variance, $B = 45.28$, $p < 0.001$) and the PO datasets ($B = 26.16$, $p < 0.001$) but not the HS datasets ($B = 0.70$, $p = 0.705$). The highest variance for all

estimators was for the HS dataset (Table 2.7). There were significant correlations between all comparisons but coefficients were consistently higher between QG and WG than between comparisons involving LR (Table 2.8). This was especially true for comparisons involving HS and PO datasets.

Table 2.7: Mean relatedness ± variance for three relatedness estimators and below and results of t-tests for deviation from expected values. The estimator with the lowest variance in each category that did not show significant bias is in bold.

	UR (n = 125)	HS (n = 38)	PO (n = 125)
QG	**-0.018 ± 0.027**	0.265 ± 0.047	0.491 ± 0.011
	t = -1.20 p = 0.233	t = 0.42 p = 0.679	t = -0.96 p = 0.337
WG	-0.015 ± 0.030	0.269 ± 0.039	**0.499 ± 0.009**
	t = -0.99 p = 0.322	t = 0.58 p = 0.569	t = -0.06 p = 0.950
LR	-0.022 ± 0.012	**0.275 ± 0.036**	0.485 ± 0.027
	t = -2.24 p = 0.027	t = 0.80 p = 0.429	t = 0.99 p = 0.324

Table 2.8: Pearson's correlation coefficients between pairwise relatedness estimators. All were significant at $p < 0.001$.

	UR	HS	PO
QG - WG	0.891	0.929	0.905
QG - LR	0.791	0.459	0.503
WG - LR	0.753	0.506	0.483

2.5 Discussion

In this study, over 400 non-duplicate grey seal adults and over 200 pups were genotyped for up to 11 microsatellite loci. No loci deviated significantly from Hardy-Weinberg equilibrium, and loci showed no significant linkage disequilibrium. These are both assumptions that are required for calculating unbiased estimates of relatedness and genetic diversity. Allen et al. (1995) reported that two loci (Hg4.2 and Hgdii) deviated significantly from Hardy-Weinberg equilibrium in the North Rona colony. They suggested the observed homozygote excess might have been due to null alleles. However, this was not found by Worthington Wilmer et al. (1999) in their larger dataset, or in this study (Table 2.4).

Error rates were 1.0% per locus, which is comparable to that found in other studies (Marshall et al. 1998; Ewen et al. 2000). Although over half of the errors were found in two loci, this was due to a variety of causes and did not appear to be caused by a

systematic problem (e.g. consistent allelic dropout or null alleles; Table 2.6). Error rates between genotypes from this lab and the Cambridge lab were 0.9% with no systematic differences in genotyping. Therefore, data from the two labs were combined for analyses in Chapters 3 and 4.

A comparison of the two types of allele assignment from fragment traces (by eye and by an automatic binning program) suggested samples typed using the binning program contained fewer errors. As the program automatically transfers genotypes to a spreadsheet, this removes copying errors. However, as the binning program was implemented later in the study, this difference may be largely due to greater expertise with lab protocols and interpreting fragment traces. Since most errors were not identified as being associated with binning but were unknown (Table 2.6), greater expertise is likely to be the main explanation.

In total, 25% of all female samples were resampled females that were removed from the final dataset. Genetic matching identified 14% as being replicate samples while the remainder were identified first using pelage. Similarly, 24% of male samples were replicates but only 2% (1 pair) was found to be a resampled male through genetic matching. As both females and males show a high degree of fidelity to breeding sites (Pomeroy et al. 1994; in press; Twiss et al. 1994), and females were often randomly sampled in multiple years, the number of resampled individuals is not surprising. Furthermore, most of the sampling, except 1997, was concentrated in one region of the colony, making resampling even more likely.

In two instances, mothers' genotypes did not match those of their pups, even after the samples were rerun. However, when the females exchanged pups, the genotypes matched for at least one allele at each locus. The pups were sampled on the same day within 10 m of each other and so the correct mother may have been difficult to identify. While fostering does occur in grey seals, it seems most likely that the two pup samples were mislabelled either in the field or the lab. It is unlikely that the two female samples were swapped, as they were sampled in different years. Otherwise, all pairs sampled as mothers and pups matched. This is surprising since Worthington Wilmer et al. (1999) found 15% of observed mother-pup pairings on North Rona were nonfilial matches using genetic markers. Worthington Wilmer et al. (1999) sampled more pups from

outside the Study Area and it is possible that fostering rates differ between regions on the colony. Also, some pairs in this study were under observation and the stage of the pup at least was known when sampled, potentially reducing human error in selecting the wrong pup.

The results of the comparison between relatedness estimators was inconclusive. Each estimator had the lowest variance for one dataset, when those that differed from the expected value were removed (Table 2.7). Highest variances were seen with the half sibling dataset, which is probably due to smaller sample sizes. It is likely that some full siblings were included in this dataset, but although all estimates were higher than the expected 0.25 they were not significantly so, suggesting this had little effect. The Lynch & Ritland (1999) relatedness estimate for the unrelated dataset was significantly lower than zero (Table 2.7). This estimator also had considerably higher variance for the parent-offspring dataset than the others. This is consistent with results from data simulated from real population allele frequencies (Van de Casteele et al. 2001; Krützen et al. 2002) and is likely to be an inherent problem with this method (Wang 2002). Thus, the Lynch & Ritland (1999) estimator was considered to be the least suitable estimator for this population.

There was little difference in variance between the Queller & Goodnight (1989) and Wang (2002) estimators (Table 2.7) and they were very highly correlated (Table 2.8). Previous studies on grey seals at North Rona have used the Queller & Goodnight (1989) estimator (Pomeroy et al. 2001; Redman 2002). It is also more commonly used in studies of kinship and population genetic structure (e.g. Carlsson et al. 1999; Burland et al. 2001; Baglione et al. 2003). Therefore, in the interest of comparability between studies, the Queller & Goodnight (1989) estimator was used throughout this thesis.

As predicted, the variance in relatedness estimates decreased when the number of loci was increased from seven to 11 (Figure 2.1). While the addition of even more loci would undoubtedly have decreased the variance further, this had to be weighed against the time and money involved in genotyping more loci. In general, the level of accuracy obtained using about ten reasonably variable loci should be sufficient to confidently differentiate between related and unrelated individuals (Blouin 2003), which was the case here (Figure 2.1B). For some analyses in this thesis, a cut off point between related

and unrelated pairs was needed. A relaxed cut off was chosen at one standard deviation above the mean of the unrelated dataset or 0.155. This includes virtually all parent offspring pairs, and ~ 71% of half sibling pairs but also includes ~ 18% of unrelated pairs. A strict cut off point was also chosen at two standard deviations above the mean of the unrelated dataset or 0.314. This has the advantage of excluding all but ~ 2% of unrelated individuals although ~ 65% of half siblings and ~ 4% of parent offspring pairs are excluded. These cut offs were chosen to balance the effects of type 1 and type 2 errors. Type 1 errors will be higher when the relaxed cut off is used, as it will contain more unrelated pairs. Conversely, type 1 errors will be low when the strict cut off is used, while type 2 errors will be higher. This may be more appropriate in situations when it is important to only have relatives in the dataset, even if some relatives are excluded.

For all relatedness estimates calculated in this thesis, background allele frequencies were calculated from adult grey seals sampled on North Rona during the breeding season. While Queller & Goodnight (1989) specify that allele frequencies be calculated from non-relatives only this is obviously impossible for studies of populations with unknown pedigrees. However, the large number of genotypes (> 400) used to calculate the population allele frequencies should provide a reasonable approximation. An alternative is to use allele frequencies from a second population, such as the Orkneys or the Isle of May, which should not include any of the North Rona animals. Using only North Rona allele frequencies will result in relatedness values for the colony being normally distributed around zero. Using allele frequencies from elsewhere will increase this value and may aid in detecting patterns through the colony. However, using allele frequencies from a second colony is likely to bias relatedness estimates, especially if they are genetically differentiated, as are North Rona and the Isle of May (Allen et al. 1995). For instance, a rare allele in the other colony that is common on North Rona will result in pairs sharing that allele to have inflated relatedness values. Therefore, due to the large number of genotyped North Rona adults and the potential bias created by using allele frequencies from another colony, all allele frequencies used to estimate relatedness were calculated from North Rona adults only.

In conclusion, a dataset was constructed of 663 non-duplicate grey seal genotypes from the North Rona breeding colony that has the properties necessary to calculate unbiased relatedness and genetic diversity estimates. These will be used throughout the following chapters. The Queller & Goodnight (1989) relatedness estimator was chosen as the most suitable for this population due to lower variances and previous use with grey seals. A relaxed and a strict cut off between related and unrelated pairs were identified.

Appendix 2.1: Allele frequencies of 11 microsatellite loci from adult grey seals (n = 412) breeding in the North Rona colony from 1997 to 2002.

Locus	Allele	Freq.	Locus	Allele	Freq.	Locus	Allele	Freq.
Hg3.6	85	0.1966	Hg8.9	199	0.0765	Pv11	158	0.0425
	89	0.0449		201	0.0437		160	0.0133
	91	0.0182		203	0.0158		162	0.0376
	93	0.0947		205	0.0061		164	0.3641
	95	0.0558		207	0.0740		166	0.3811
	97	0.2561		209	0.2682		168	0.0971
	99	0.3131		211	0.1990		170	0.0570
	101	0.0206		213	0.1371		172	0.0073
				215	0.0243			
Hg4.2	141	0.1034		217	0.1420	Hl15	105	0.1201
	153	0.5693		219	0.0133		113	0.1165
	155	0.1849					131	0.0546
	157	0.0049	Hg8.10	181	0.0232		133	0.0522
	159	0.0925		183	0.0951		135	0.0801
	161	0.0219		185	0.1159		137	0.2840
	163	0.0170		187	0.3390		139	0.0570
	165	0.0012		189	0.2512		141	0.0133
	167	0.0036		191	0.0390		143	0.0352
	169	0.0012		193	0.0268		145	0.0534
				195	0.0683		147	0.0109
Hg6.1	150	0.0279		197	0.0085		149	0.0206
	156	0.0255		199	0.0329		151	0.0146
	160	0.0631					153	0.0643
	162	0.4842	Hgdii	185	0.0777		155	0.0158
	164	0.3811		199	0.4648		157	0.0049
	166	0.0182		201	0.0255		159	0.0024
				207	0.0085			
Hg6.3	223	0.0280		209	0.0862	Lc6	228	0.0162
	225	0.1610		211	0.2985		234	0.0037
	227	0.3073		213	0.0328		236	0.1356
	229	0.2366		215	0.0061		238	0.2587
	231	0.0878					240	0.0498
	233	0.1793	Pv9	162	0.0218		242	0.0398
				164	0.2706		244	0.0522
				166	0.2136		246	0.1032
				168	0.1833		248	0.3022
				170	0.0291		250	0.0211
				172	0.1396		252	0.0174
				174	0.1420			

Chapter 3: Fine-scale spatial and temporal patterns of genetic relatedness in breeding female grey seals, *Halichoerus grypus*

3.1 Introduction

The spatial and temporal distribution of kin within a population can influence social behaviour and mating patterns. If interactions among close relatives are common, due to a non-random distribution of kin, then social behaviours that increase inclusive fitness can evolve through kin selection (Hamilton 1964, reviewed in Hughes 1998; Ross 2001). Kin clustering can also increase the probability of inbreeding, and thus behaviours that minimise mating between relatives are likely to evolve (Sugg et al. 1996). Therefore, knowledge of kin distributions within a population can help to predict and understand behavioural processes which, may in turn, lead to a greater understanding of differences in individual fitness.

Fine-scale clustering of kin can occur passively, through high natal site fidelity, or actively, due to a preference for interacting with kin. Passive kin clustering occurs when individuals prefer their natal site, where they may benefit from local experience or familiarity with neighbours and may have access to higher quality resources (Johnson & Gaines 1990; Part 1991; Schjørring 2001). Alternatively, individuals may actively prefer to interact with kin if they can gain inclusive fitness benefits. This requires a type of recognition mechanism that allows for reliable identification of relatives. Behavioural rules-of-thumb, such as familiarity, may be relied upon if those individuals that associate for a certain period (e.g. nest mates) are likely to be kin (Holmes 1988; Komdeur & Hatchwell 1999). Alternatively, phenotype matching may be used. Here individuals compare their phenotype, or that of a known relative, to the phenotype of an unknown individual; the similarity of phenotypes must be strongly correlated with relatedness level (Sherman et al. 1997). Odour cues, often associated with the MHC complex, are thought to be important in phenotype matching (Arcaro & Eklund 1999; Mateo 2002; Olsson et al. 2003), although visual and vocal cues may also be used (Price 1999; Arnold 2000).

Kin clustering has been observed in a wide variety of species. Cooperatively breeding groups, usually found in birds, primates and carnivorous mammals, are almost always composed of highly related individuals (Emlen 1997). Natal dispersal is often sex biased and in general, males tend to be the dispersing sex in mammals (Greenwood

1980). Thus, many mammalian social groups are composed of female kin, such as those formed by European wild rabbits, *Oryctolagus cuniculus*, (Surridge et al. 1999) and Bechstein's bats, *Myotis bechsteinii* (Kerth et al. 2000). Kin clustering has also been observed in species with poorly developed social systems. For example, Coltman et al. (2003), found genetic structuring between hefts within a population of Soay sheep (*Ovis aries*). Female relatives within hefts also tended to cluster together due to incomplete natal dispersal. In seabird breeding colonies, several studies have found relatives nest close to each other as a result of high natal site fidelity (Schjørring 2001) and preference for familiar individuals (van der Jund et al. 2002). Finally, solitary mammals have been found to live close to and interact more with relatives, probably due to natal philopatry (Ratnayeke et al. 2002).

Kin clustering may be beneficial if it results in an increase in an individual's inclusive fitness. This has been widely studied in breeding groups where cooperative behaviours, such as helping to rear offspring, are thought to have evolved through kin selection (Emlen 1997). In some instances though, seemingly altruistic behaviour of individuals in social groups can be explained by direct fitness benefits alone (Dunn et al. 1995; Clutton-Brock et al. 1999; Griffin & West 2002). Bird leks are another system where kin clustering has been found to increase participants' inclusive fitness (Petrie et al. 1999; Shorey et al. 2000). Males that cluster with kin gain indirect fitness benefits by increasing the size of a lek that contains successful relatives, as this leads to more overall matings. Kin clustering is beneficial to some territorial species as well, with reproductive success increasing when close relatives hold neighbouring territories (e.g. male red grouse, *Lagopus lagopus scoticus*, Piertney et al. 1999; female voles, *Microtus spp*, Lambin & Yoccoz 1998; Pusenius et al. 1998). However, in territorial salmonids, kin clustering does not generally occur (Fontaine & Dodson 1999), despite evidence of beneficial kin-biased behaviour (Brown & Brown 1993; Griffith & Armstrong 2002).

No evidence of kin clustering has been found in studies of phocid breeding colonies (Perry et al. 1998; Schaff et al. 1999; Pomeroy et al. 2001) despite site fidelity and philopatry being characteristic of some species (Pomeroy et al. 2000a; Härkönen & Harding 2001; Kretzmann et al. 2001). In lactating female harbour seals, *Phoca vitulina*, Schaeff et al. (1999) found no correlation between the degree of relatedness and the time spent in the same group. Furthermore, no genetic structure was apparent

between female grey seals pupping on different beaches within island colonies (Perry et al. 1998). Both of these studies used relatively small sample sizes and multilocus DNA fingerprinting, which are less useful than microsatellites in resolving relatedness (Bruford et al. 1992). However, a preliminary study, using microsatellites, also found no evidence of kin clustering in breeding female grey seals (Pomeroy et al. 2001).

In this study, the fine-scale genetic structure of female grey seals breeding in the North Rona colony was examined. Evidence for strong fidelity of adult females to previous pupping sites and dates (Pomeroy et al. 1994; 1999; in press), site fidelity of males (Twiss et al. 1994), philopatry to the colony (Allen et al. 1995; Pomeroy et al. 2000a), and natal site fidelity within the colony (Pomeroy et al. 2000a), suggest kin clustering may be present. The North Rona breeding colony is a temporary aggregation that forms every year in the autumn and lasts for approximately ten weeks, with individual females suckling their pups for an average of 17 days before returning to sea (Pomeroy et al. 1999). Median female fidelity to previous pupping sites in consecutive years has been recorded as 55 m for branded females (Pomeroy et al. 1994) and more recently as 39 m for a larger sample of females, including many that were identified from pelage markings only (Pomeroy et al. in press). Individual females also tend to pup at the same time during the season, to within a few days of previous pupping dates (Pomeroy et al. 1999; in press). However, philopatry estimates have been difficult to obtain for grey seals. Using tagged pups, Pomeroy et al. (2000a) estimated the rate of female philopatry to be only 0.36 on North Rona, but this measure was confounded by a low sample size due to high tag loss rate and pup mortality. Nevertheless, the individuals that returned to the colony pupped nearer to their birth site than expected.

Genetic differentiation between UK colonies also suggests that many grey seals are philopatric and gene flow is limited (Allen et al. 1995). However, the significant F_{ST} estimate found by Allen et al. (1995) was low (F_{ST} = 0.006) and compared the North Rona and Isle of May colonies, which are separated by about 500 km. Other studies examining grey seal colonies in closer proximity to each other have failed to find significant genetic differentiation (Boskovic et al. 1996; Gaggiotti et al. 2002; Graves et al. submitted).

3.2 Aims

The aim of this chapter was to examine spatial and temporal patterns of genetic relatedness between female grey seals pupping in the North Rona breeding colony. Genetic structuring was tested between distinct predefined regions within the colony. Since females are generally faithful to the predefined regions (Pomeroy et al. 1994), genetic differentiation between regions was predicted. Evidence for spatial and temporal kin clustering was also tested within regions. Females that pupped closer together were predicted to be more related than the rest of the colony. This was also investigated by examining the spatial and temporal distribution of related versus unrelated pairs. It was predicted that related pairs would pup closer together in time and space.

In addition, genetic matches were used to estimate median adult female fidelity to pupping sites and dates. Genetic matches occurred when two samples, taken in different years, had identical genotypes (and were therefore the same female). These individuals had not previously been identified by brands or pelage markings and should provide an alternative method of estimating site fidelity.

3.3 Methods

3.3.1 Sampling

This chapter used females that were recorded in the colony in 1997 and 2000 to 2002 (see Chapter 2). Females were only included in analyses if they were seen with a pup in that year. Samples were genotyped for 11 microsatellite loci as described in Chapter 2 and only those genotyped at seven or more loci were included. The 1997 data set was supplemented by 96 samples genotyped in Bill Amos's lab in Cambridge. These samples were genotyped for nine of the same loci (not Hl15 and Lc6) and were directly comparable to the other genotypes used here (Chapter 2).

In 1997, mothers were sampled from the three main pupping regions in the North Rona colony: the Study Area (SA), Fianuis South (FS), and Fianuis North (FN) (Figure 3.1). These regions have been identified based of the location of access gullies to the sea and the distribution of females (Figure 3.1; Pomeroy et al. 1994). Only a few mothers from outside SA were sampled in 2000 (n = 17), 2001 (n = 10) and 2002 (n = 14) and these

were removed from all analyses, as sample sizes were too small to produce meaningful results.

The location of each sampled mother was recorded in the field on a detailed geo-rectified map overlaid with a 10 m interval Ordinance Survey grid (Twiss et al. 2001). The location recorded was the pupping location of the mother or her location when first seen with a pup, as observed from hides overlooking the colony. When both of these were not available sampling location was used. In 1997, location was recorded as the 10 m^2 grid cell in which a female pupped, whereas in all other years locations were recorded to within 2 m accuracy. In order to standardize between years, locations in 2000 to 2002 were subsequently binned into the appropriate 10 m^2 grid cell. As mothers typically remain within 10 m of their pupping site (Pomeroy et al. in press) most mothers should have been placed in the cell in which they pupped.

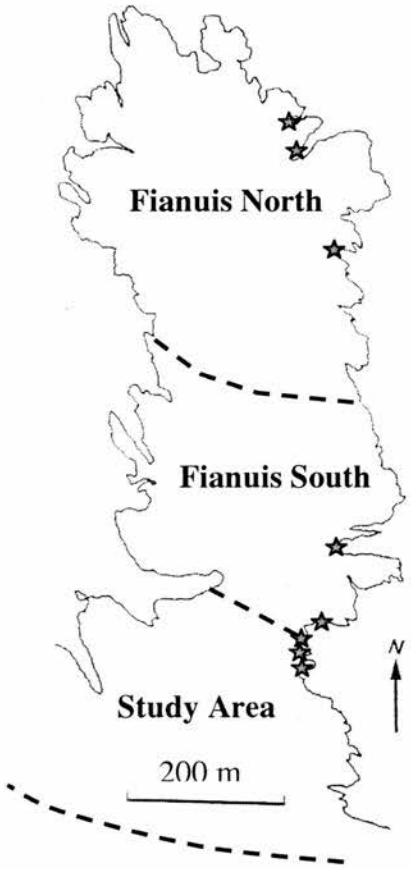


Figure 3.1: The Fianuis peninsula, North Rona, showing divisions between the three regions used in this thesis. Stars indicate the locations of access gullies to the sea (modified from Pomeroy et al. 1994).

Pupping dates were recorded using September 01 as day 1. Pupping date could be recorded directly if mothers were seen giving birth or had been observed the previous day without a pup. Otherwise, pupping date was estimated from the sampling date by subtracting the estimated age of the pup. Pups were divided into five stages using a standard classification system based on appearance, with pup age taken as the average age of pups at each stage (Table 3.1). Since there is greater variation in the age of pups at later stages, the later the stage of the pup when sampled, the lower the accuracy of the pupping date (Redman 2002). Some females were observed repeatedly with pups, but observation began when the pup was already present. In these instances, pup age could be estimated more accurately by including information about the length of time a pup remained at each stage.

Table 3.1: Average age of pups at each pup stage and a description of the characteristics used to classify pups in the field (Pomeroy, pers. comm.; after Kovacs and Lavigne 1986).

Pup Stage	Average Age (d)	Brief description
I	1	Yellow tint to pelage, fresh umbilicus present, outline of hips and ribs clearly visible
II	6	White pelage, shoulder to hip region filled out, ribs not visible
III	11	Barrel shaped body, no moulting, all white
IV	15	Lanugo moulting, patches of juvenile pelage showing through
V	20	Completely moulted

From 2000 to 2002 many females could be identified from pelage markings, and therefore pupping locations and dates could be recorded from hides overlooking the colony and, if necessary, mothers could be sampled at a later date. In 1997 this was only possible for branded females (11 out of 245) as a system of pelage identification was not in place and many samples were taken from locations that were not visible from the hides (i.e. all samples from FN). As a result, 82% of mothers in 1997 had pups greater than stage I when sampled (or first observed) compared with 45% in 2000, 30% in 2001 and 17% in 2002. This resulted in differences between years in the accuracy of estimates.

The distribution of samples differed spatially and temporally between years. This was due to both the actual distribution of mothers differing between years and differences in sampling, as the dataset represented only a subset of females present in the colony. To visualise differences in female distribution between years, plots of the number of

females using each 10 m^2 grid cell over the season were created (by S. Twiss). Locations of all females on the colony visible from hides (i.e. SA and most of FS only) were recorded each day on detailed geo-rectified maps overlaid with a 10 m interval Ordinance Survey grid (by P. Pomeroy). These were entered into an ARC-INFO GIS of the North Rona colony. The number of females in each cell was estimated by taking the cumulative number of females present (measured daily) and dividing by 18, the average length of stay of mothers, in days. For each year, the season was defined as the first day data were available to the last day a pupping date was recorded. The proportion of mothers within each cell that were sampled was also plotted to visualise sampling intensity. Pupping dates of sampled mothers were also compared between years and within regions using Kruskal-Wallis tests, as the data were not normally distributed.

3.3.2 Site and temporal fidelity estimates

Site fidelity of females pupping in the North Rona colony has been estimated in previous studies (Pomeroy et al. 1994; in press; Redman 2002) and many of the mothers used here form part of a current study on site fidelity (Pomeroy unpublished). In this study, site and temporal fidelity were estimated only for mothers that were identified in two or more years based on genotyping alone. Only matches involving one sample being taken in 1997 and the other in either 2000, 2001 or 2002 were used. This was because from 2000 to 2002 many females were identified by pelage markings and thus few unknown genetic matches occurred between years (n = 18), making sample sizes small for each year pair. Additionally, samples were biased towards mothers that were highly site faithful as sampling was concentrated in the same areas of the colony in 2000 to 2002. This meant that mothers that moved larger distances between years were less likely to have genetic samples taken in two different years. In 1997 samples were taken from the entire colony and thus comparisons should not suffer from this bias. Site fidelity was measured in meters by taking the shortest distance between a mother's pupping sites. First, the median distance between sites was calculated for 1997-2000, 1997-2001 and 1997-2002 separately. As some mothers were present in three or more years, a site fidelity estimate for each mother was determined by taking the median of all her site fidelity estimates. To obtain an overall measurement the median of site fidelity estimates for all mothers was taken.

To get an estimate of how consistent mothers were with respect to their pupping date, the difference between dates for each mother was taken. Comparisons were made between 1997-2000, 1997- 2001 and 1997- 2002 and combined as described for the site fidelity estimates above. All statistical analyses in this section were performed in SPSS 11.0.

3.3.3 Genetic differentiation of regions

To investigate genetic structuring in the colony it was divided into i) north and south with the divide between FS and FN and ii) into the three regions (SA, FS, FN; Figure 3.1). Data for these analyses were available for 1997 only, as only SA was sampled in all other years. Genetic differentiation of these regions was tested using F_{ST} (following Weir & Cockerham 1984) and R_{ST} (following Michalakis & Excoffier 1996). F_{ST} uses an infinite allele model of mutation while R_{ST} uses allele sizes instead of allele identity (Slatkin 1995; Rousset 1996). This may be a more appropriate model for microsatellite data as it is based upon the stepwise model of mutation (Kimura & Ohta 1978). The statistical significance of F_{ST} and R_{ST} scores was tested using 10 000 permutations of individuals among regions using SPAGeDi 1.1 (Hardy & Vekemans 2002). Allele frequencies were compared between regions in GENEPOP 3.3 (Raymond & Rousset 1995). A Fisher exact test was used to determine statistical significance for each locus and for all loci combined. Average pairwise relatedness between regions and within regions was also estimated, using the Queller & Goodnight (1989) relatedness estimator (as described in Chapter 2) in SPAGeDi 1.1 (Hardy & Vekemans 2002). To test for significance, a dataset of pairwise relatedness values of all females (n = 455) was created and 1000 values were randomly selected and the mean taken. This was repeated 1000 times, which resulted in a distribution with a mean of 0.000 ± 0.006 SD. Mean relatedness estimates of females within and between regions were considered significantly different from this mean of zero if they fell outside of 1.96 SD of the mean. For all estimates of relatedness, background population allele frequencies from 499 adult males and females sampled in the North Rona colony between 1997 and 2002 were used.

3.3.4 Fine-scale patterns of relatedness

To examine fine-scale spatial and temporal patterns of relatedness, data from 1997, 2000, 2001 and 2002 were used. The 1997 data were analysed separately for each

region in order to allow comparisons with other years. All distances between individuals were calculated as Euclidean distances. Correlations of matrices of pairwise relatedness and distance apart (in meters) were performed to test for spatial structuring. The average pairwise relatedness of neighbours was then compared to the average relatedness of all other pairs of mothers within the region. All mothers inside the same 10 m^2 grid cell and the eight surrounding grid cells were chosen to represent neighbours. As mothers typically remain within 10 m of their pupping site (Pomeroy et al. in press), mothers in more distant grid cells are unlikely to interact during the season. For the above tests, statistical significance was tested using 10 000 permutations of individual locations among individuals. This is similar to a Mantel test except that the locations of individuals are permutated not the rows and columns of the distance matrix.

To test for temporal aggregations of kin, correlations of pairwise relatedness and difference in pupping date (in days) were performed. Average pairwise relatedness of mothers pupping within nine days of each other was compared to those pupping greater than nine days apart. As mothers suckle their pups for an average of 17 days, the nine day interval corresponds to pairs overlapping for more than half their breeding time. For temporal analyses, statistical significance was tested using 10 000 permutations of rows and columns of the date matrix, as in a Mantel test.

Standard errors for mean pairwise relatedness estimates were estimated by jackknifing over loci. Permutation tests did not take into account missing data and so may be biased for loci that were not genotyped for many individuals. This is the case for two loci (Hl15 and Lc6) in 1997 and so analyses were repeated with these loci removed to ensure they were not unduly influencing the result. All fine-scale analyses described above were performed using SPAGeDi 1.1 (Hardy & Vekeman 2002).

Fine-scale patterns of relatedness were also investigated by taking an alternative approach and comparing the distance (both spatial and temporal) between related and unrelated pairs. First, relatives were defined as those pairs with a relatedness value of 0.155 or greater, which should include most half siblings and all parent-offspring and full sibling pairs (relaxed cut off definition, described in Chapter 2). The analyses were then repeated with related pairs being defined as those with a relatedness value 0.314 of greater (strict cut off definition, described in Chapter 2). This definition will exclude

most unrelated pairs but also many half sibling pairs. The strict definition of relatedness was used since the large number of unrelated pairs included in the relaxed definition might obscure a signal from related pairs. In addition, trends may be most apparent in first-order relatives, which would be more readily detected using the strict definition. Distances between pupping sites of related and unrelated pairs were compared using Mann-Whitney U tests for all years and regions. As distances between mothers are pairwise and therefore not independent, significance was tested using 10 000 Monte Carlo randomisations of distances among groups in SPSS 11.0.

3.4 Results

A total of 405 mothers were genotyped over the four years for at least seven loci (1997 n = 245; 2000 n = 73; 2001 n = 136; 2002 n = 121). In the 2000 to 2002 datasets, all mothers were typed for 11 loci with the exception of one mother, present in both 2001 and 2002, that was typed for only 10 loci. In 1997, only 60% (150/245) of samples were typed for all 11 loci and two loci (Hl15 and Lc6) were missing from 35% (85/245) of samples.

3.4.1 Distribution of samples

There was some variation in the locations used by females in the colony in the years studied (Figure 3.2). For example, females used the central area of SA less in 2002 than other years. Females used the fewest 10 m^2 grid cells in 2000 (n = 101), the most in 2001 (n = 187) and an intermediate number in 1997 (n = 135) and 2002 (n = 133). The number of high density grid cells (> 3 females) was higher in 2001 (n = 16) and 2002 (n = 14) than in 1997 (n = 7) and 2000 (n = 6). Therefore, there was variation between years in the distribution and density of females on the colony.

The highest proportion of females was sampled in the southern part of SA in all years (Figure 3.3). Within this area, at least one female was sampled in over half of used grid cells in all years, with the greatest proportion being sampled in 2002 (77%) and the least in 1997 (52%). Females in the northern part of SA were rarely sampled as this contained crowded access gullies where sampling caused high levels of disturbance. Females were only sampled in FS in 1997.

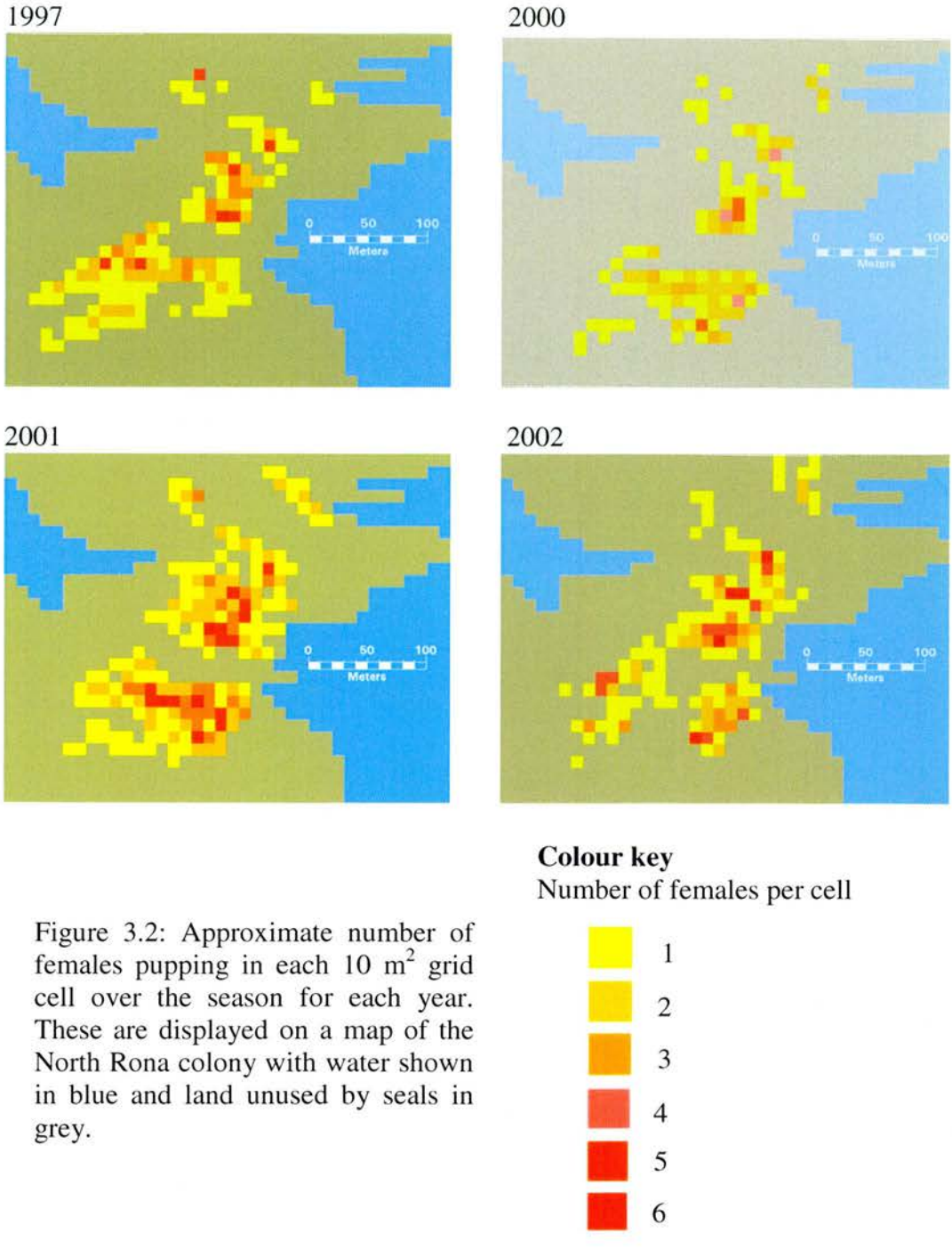


Figure 3.2: Approximate number of females pupping in each 10 m^2 grid cell over the season for each year. These are displayed on a map of the North Rona colony with water shown in blue and land unused by seals in grey.

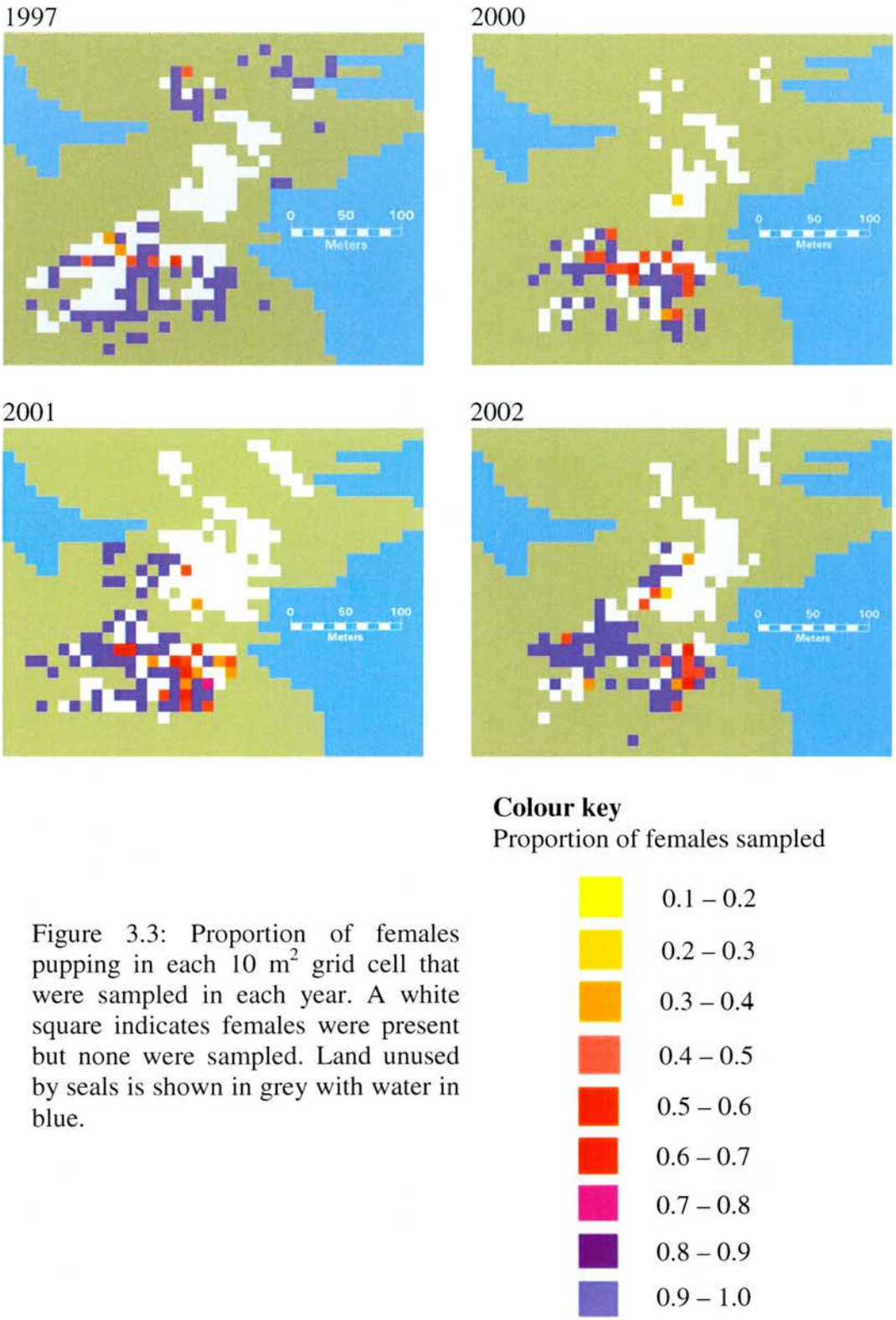


Figure 3.3: Proportion of females pupping in each 10 m^2 grid cell that were sampled in each year. A white square indicates females were present but none were sampled. Land unused by seals is shown in grey with water in blue.

Pupping dates of sampled females were distributed normally in 2000 to 2002 but not within regions in 1997 (Table 3.2). There were significant differences between pupping dates of sampled females in SA between years (Kruskal-Wallis $\chi^2 = 9.94$, d.f. = 3, p = 0.009). Non-parametric post hoc tests (following Siegel & Castellan 1998) indicated the significant differences were between 2000 – 2001 and 2000 – 2002. This is likely to be due to a lack of sampled mothers with pupping dates near the end of the season in 2000 compared with other years (Table 3.2). There were also significant differences between regions in 1997 (Kruskal-Wallis $\chi^2 = 73.12$, d.f. = 2, $p < 0.001$). Females in FN had significantly earlier pupping dates than those in the SA or FS but there was no significant difference between pupping dates in the SA and FS (Non-parametric post hoc tests, following Siegel & Castellan 1998).

Table 3.2: Descriptive statistics of pupping dates for sampled females, recorded using September 01 as day one. Deviation from the normal distribution was tested using Kolmogorov-Smirnov (K-S) tests. Significant results are highlighted in bold.

Year	Region	n	K-S Z	p	Median	Minimum	Maximum
1997	SA	106	**1.87**	**0.002**	43	28	54
1997	FS	45	**1.53**	**0.019**	41	29	54
1997	FN	94	**1.74**	**0.005**	34	25	47
2000	SA	73	0.94	0.275	40	23	54
2001	SA	136	0.81	0.501	43	22	67
2002	SA	121	0.74	0.396	43	29	66

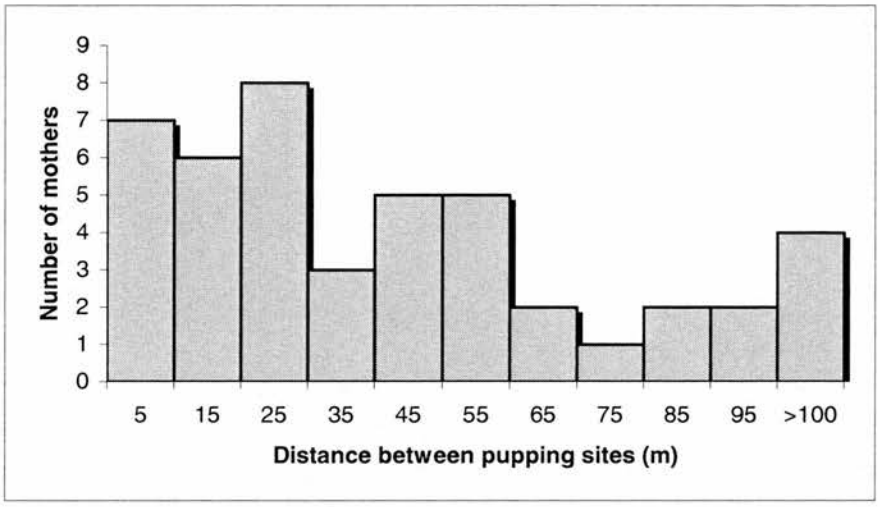
3.4.2 Site and temporal fidelity

Site fidelity estimates for separate year comparisons increased with the difference in time between years (Table 3.3) however, differences between year comparisons were not significant (Kruskal-Wallis, $\chi^2 = 0.56$, d.f. = 2, p = 0.755). There were 17 mothers with two site fidelity estimates and nine mothers with three site fidelity estimates in different year comparisons. When estimates for mothers were combined the median site fidelity estimate rose slightly indicating less fidelity (Table 3.3). The overall distribution of site fidelity was right skewed (Figure 3.4A) with only seven mothers (out of 45) having a median site fidelity estimate of 10 m or less.

Table 3.3: Median site and temporal fidelity estimates with interquartile ranges (IQR) between 1997 and each of 2000, 2001 and 2002 and an overall comparison.

		Site fidelity (m)		Date fidelity (d)	
	n	**Median**	**IQR**	**Median**	**IQR**
1997 - 2000	21	32	20 - 61	4	3-8
1997 - 2001	27	36	22 - 64	5	1-8
1997 - 2002	31	41	22 - 70	4	3-8
All	45	42	22 - 66	4	3-7

A



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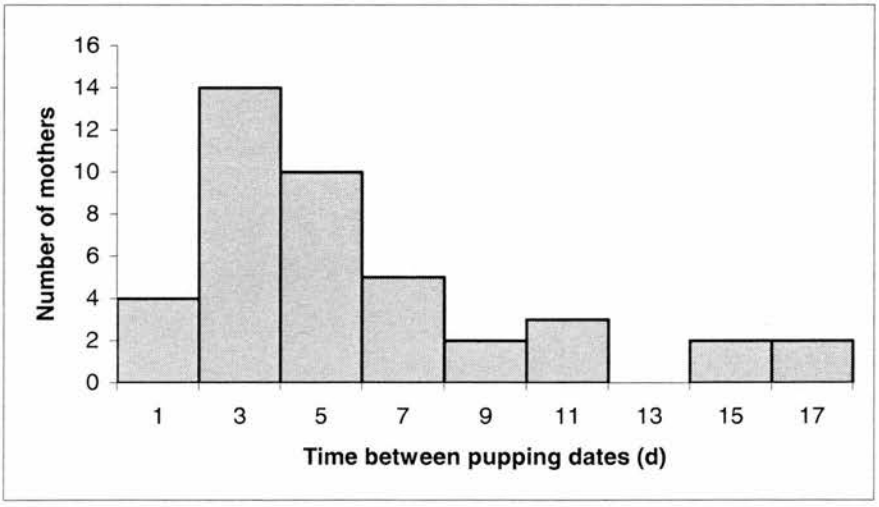


Figure 3.4: Distribution of combined A) site fidelity and B) temporal fidelity estimates of mothers (n = 45).

Females tended to be consistent between years in their pup date (Table 3.3) and there were no differences between estimates for different year comparisons (Kruskal-Wallis test, $\chi^2 = 0.45$, d.f. = 2, p = 0.797). When temporal fidelity estimates from all mothers

were combined, the distribution of estimates was right skewed (Figure 3.4B). Over half of all females pupped within a median of six days of previous pupping dates. The maximum number of days between pupping dates for a female was 17, approximately half of the length of the observed breeding season.

3.4.3 Genetic differentiation of regions

There was no evidence of genetic differentiation within the North Rona colony when it was divided into north and south (F_{ST} = 0.001, p = 0.230; R_{ST} = -0.021, p = 0.827) or three regions (F_{ST} = 0.002, p = 0.160; R_{ST} = -0.023, p = 0.787). Allele frequencies between regions were also not significantly different (north and south, χ^2 = 25.05, d.f. = 22, p = 0.295; three regions, χ^2 = 30.61, d.f. = 22, p = 0.104). When allele frequencies of individual loci were compared between three regions there was a significant difference between frequencies at only one locus, Pv9 (p = 0.028), but this was not significant after Bonferroni corrections for multiple comparisons. Within regions, average pairwise relatedness of females was only significantly different from zero in FS (p < 0.01; Figure 3.5). No average relatedness values of females between regions differed from zero (Figure 3.5). This was true when the colony was divided into north and south and when it was divided into three regions.

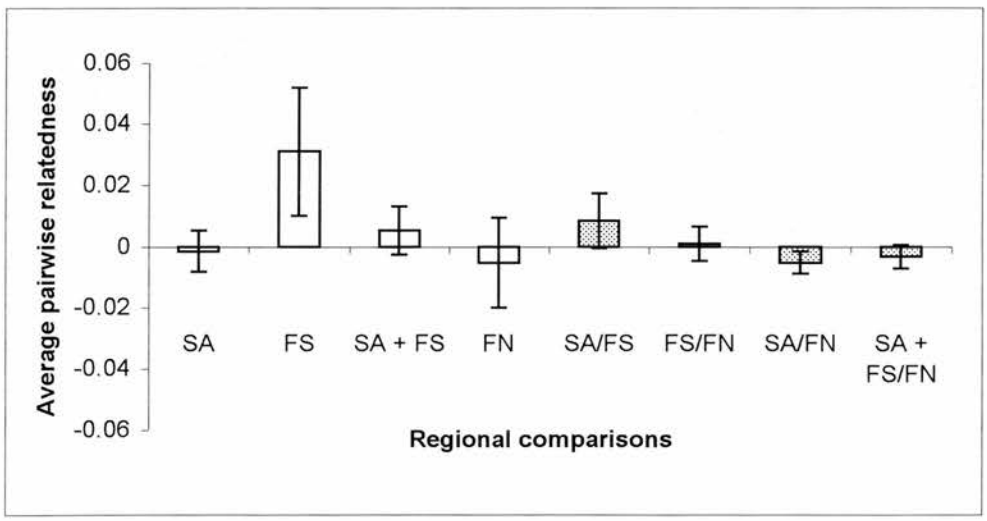


Figure 3.5: Means ± SE of pairwise relatedness values within regions (white bars) and between regions (grey bars). Plus signs between SA and FS indicate that these were combined to make the southern region. Slash marks between regions indicate relatedness was compared between the regions.

3.4.4 Fine-scale patterns of relatedness

Pairwise relatedness of mothers decreased significantly with increasing distance between pupping sites in FS in 1997 (Table 3.4). This was the only year or region where a significant relationship between geographic distance and pairwise relatedness occurred. In 1997, average pairwise relatedness of neighbours was significantly higher than expected in the SA ($p = 0.031$) and FS ($p = 0.007$) only (Figure 3.6). However, only the FS result remained significant after Bonferroni corrections for multiple comparisons. In all other years (which include the SA region only) average pairwise relatedness of neighbours and all other pairs was close to zero and were not significantly different from expected values (Figure 3.6).

Table 3.4: Regressions between the relatedness of pairs and both their geographic distance apart and time between pupping dates. Significant values are highlighted in bold.

		Geographic distance		Pupping date	
Year	**Region**	**Slope**	**p**	**Slope**	**p**
1997	SA	1.81×10^{-5}	0.553	1.73×10^{-4}	0.587
1997	FS	$\mathbf{-4.90 \times 10^{-4}}$	**0.006**	-1.99×10^{-3}	0.244
1997	FN	-5.99×10^{-5}	0.182	1.56×10^{-3}	0.952
2000	SA	-1.27×10^{-5}	0.899	1.90×10^{-3}	0.764
2001	SA	4.76×10^{-5}	0.607	9.34×10^{-5}	0.831
2002	SA	-1.07×10^{-5}	0.858	-6.12×10^{-4}	0.222

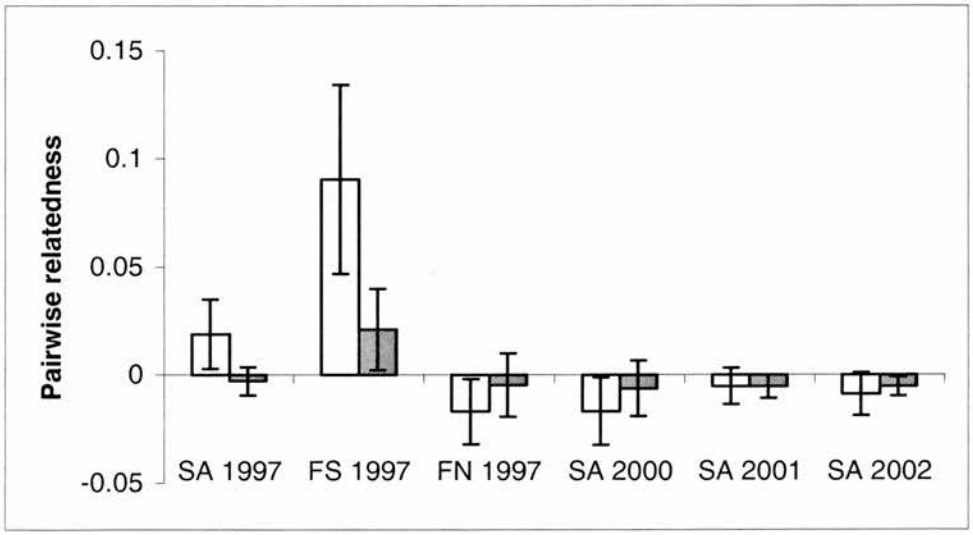


Figure 3.6: Means ± SE of pairwise relatedness of neighbours (white bars) and all other pairs (grey bars).

There was no significant relationship between pairwise relatedness of mothers and the time between their pupping dates for any region or year (Table 3.4). Average pairwise relatedness of mothers pupping both within nine days of each other and further than nine days apart were not significantly different for any region or year (Figure 3.7).

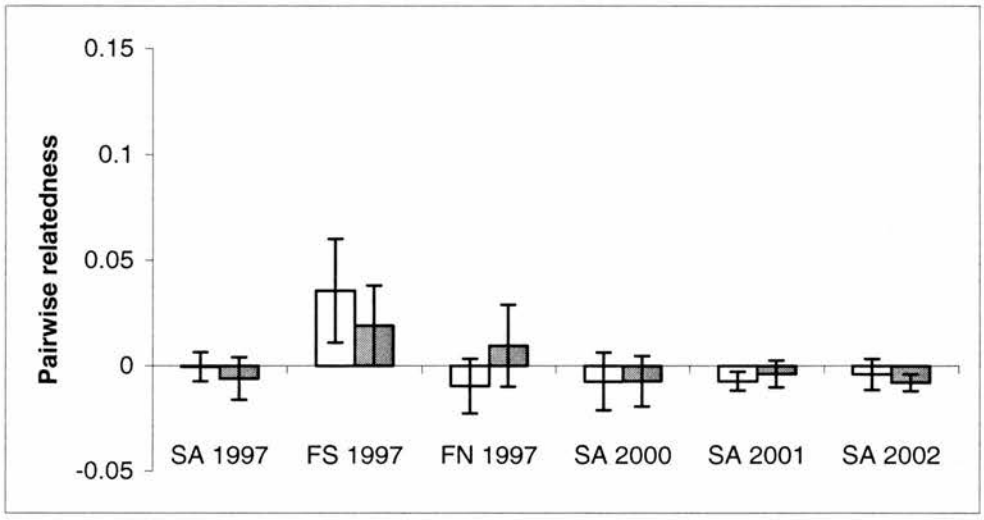


Figure 3.7: Means ± SE of pairwise relatedness of mothers pupping within nine days of each other (white bars) and pairs pupping greater than nine days apart (grey bars).

The 1997 analyses were repeated using nine loci to assess the possibility that missing data at two of the loci were biasing the significance testing. There was no change in the conclusions from any of the analyses; all significant results remained so and no trends became significant.

The distances between pupping locations of related and unrelated pairs were not significantly different in any year or region except in the FS in 1997 (relaxed relatedness $Z = -2.32$, $p = 0.016$; strict relatedness $Z = -3.17$, $p = 0.002$; Table 3.5). Only the result using the strict definition remained significant after multiple comparison testing. There were significant differences between the two groups in the number of days between pupping dates in four comparisons, two using the relaxed definition of relatedness (FS 1997 $Z = -2.54$, $p = 0.021$; SA 2002 $Z = -2.49$ $p = 0.013$) and two using the strict definition of relatedness (FN 1997 $Z = -2.44$, $p = 0.017$; SA 2001 $Z = -2.36$, $p = 0.017$). None of these were significant after multiple comparison testing. In no instance was a result significant for a year or region using both relatedness definitions. For all significant results, except SA in 2001, there was no difference in the medians of the related and unrelated group making interpretation difficult (Table 3.5).

Table 3.5: The distance apart and time between pupping dates for related and unrelated pairs of mothers using A) the relaxed definition of relatedness ($r > 0.155$) and B) the strict definition of relatedness ($r > 0.314$). Significant differences are highlighted in bold.

A

Year	Region	Number		Median distance		Median date	
		related	unrelated	related	unrelated	related	unrelated
1997	SA	956	4609	60.00	56.57	5	5
1997	FS	271	719	**72.80**	**80.62**	**6**	**6**
1997	FN	897	3474	94.87	98.49	5	5
2000	SA	429	2199	50.00	50.99	8	8
2001	SA	1517	7663	60.83	60.83	10	10
2002	SA	1222	6038	64.03	64.03	**8**	**8**

B

Year	Region	Number		Median distance		Median date	
		related	unrelated	related	unrelated	related	unrelated
1997	SA	197	5368	56.57	58.31	6	5
1997	FS	81	909	**50.00**	**80.62**	6	6
1997	FN	237	4134	90.55	98.49	**5**	**5**
2000	SA	75	2553	50.00	50.99	7	8
2001	SA	273	8907	60.83	60.83	**7.5**	**10**
2002	SA	224	7036	63.25	64.03	9	8

3.5 Discussion

As female grey seals in the UK are philopatric (Allen et al. 1995; Pomeroy et al. 2000a) and are highly faithful to previous pupping sites (Pomeroy et al. 1994; in press), some degree of genetic structuring within colonies might be expected. Yet this study found little evidence of genetic structuring of grey seal mothers in the North Rona breeding colony when it was examined at a colony-wide level and when the distribution of relatedness was investigated within regions. However, significant kin clustering was observed in one region, which is, to my knowledge, the first time it has been identified in a phocid colony. The general lack of genetic structure, though, agrees with other studies of kin distribution in grey and harbour seal colonies (Perry et al. 1998; Schaff et al. 1999; Pomeroy et al. 2001).

Adult female site and temporal fidelity were estimated in this study using mothers matched between years by their genotype only. The results are comparable to those from other studies, suggesting this is a useful method for estimating site and temporal fidelity. The overall median site fidelity estimate of 42 m is lower than that estimated using branded animals (55 m, Pomeroy et al. 1994) but is very similar to the recently

published estimate of 39 m, which used locations from over 300 mothers (Pomeroy et al. in press). Estimates from this study used female pupping locations that were up to five years apart, while other studies only used females pupping in consecutive years. The similarity of these estimates suggests that site fidelity is consistent over many years. Fidelity to pupping date was also high (Table 3.3) and similar to previously reported results (Pomeroy et al. 1999; in press).

There was no significant genetic differentiation between regions suggesting high levels of gene flow within the colony. This result was not affected by combining the Study Area and Fianuis South to make one southern region. Gene flow may be caused by adult migrants moving between regions and between breeding colonies; both of these behaviours have been observed in UK grey seals (Pomeroy et al. 2000a; Redman 2002). Furthermore, females do not always breed every year (Pomeroy et al. 1999) and may mate with males away from their usual breeding colony, causing of gene flow (Worthington Wilmer et al. 1999). Even in the absence of gene flow, the colony may appear panmictic because there has not been enough time for genetic differences to accumulate between regions. The North Rona breeding colony was founded in the 1840s (Boyd et al. 1962). Assuming a generation time of 16 years for grey seals (J. Graves, J. Harwood pers. comm.), the breeding colony is only approximately 10 generations old. Therefore, it seems likely that both gene flow and a brief period of time since separation may account for the lack of genetic structuring between regions.

Within the Study Area and Fianuis North, no significant kin clustering was detected in any year (Figure 3.6; Table 3.4). This suggests the precision with which females return to their natal site is not high enough to produce patterns of kin clustering at the scale that was studied. Natal site fidelity has been estimated to be within 100 m from the few available marked females on North Rona (Pomeroy et al. 2000a). This level of natal site fidelity would be unlikely to produce measurable kin clustering within regions, as they are each only a few hundred meters across (Figure 3.1). However, this degree of natal site fidelity should produce higher pairwise relatedness within regions than between, which was not generally found (Figure 3.5). Thus, this study suggests that natal site fidelity is lower than pupping site fidelity in female grey seals but at what scale it occurs could not be measured here.

A high degree of natal site fidelity is beneficial in many species because individuals may get access to higher quality resources at their natal site, as long as habitat quality is unchanging (Schjørring 2001; Pärt 1991). In grey seal colonies habitat quality varies, with preferred pupping sites being close to access to water with low levels of disruption by conspecifics (Pomeroy et al. 2000b; Twiss et al. 2000a; 2001). Thus, it would seem beneficial for daughters to return to their natal site, as this should be better habitat than one chosen at random. However, while some topographic characteristics are constant, locations and sizes of pools of water and the distribution of females, and therefore also males, can change between years, altering the quality of sites. Females come ashore prior to pupping for an average of two days and may use this time to evaluate pupping sites (Pomeroy et al. 1994). Females may also be unable to pup in their natal sites because of competition for high quality habitat. The most preferred sites on the colony are occupied first (Pomeroy et al. 2000b; Twiss et al. 2001) and these mothers may be older and of higher quality (Pomeroy et al. 1999; 2001; Twiss et al. 2000a). Therefore, changing habitat quality and competitive ability may influence site selection on the colony causing low natal site fidelity and an absence of kin clustering.

A lack of high natal site fidelity among one sex may also be a tactic to avoid inbreeding (Libers & von Schantz 1985; Packer 1985). During the breeding season, some males defend areas around groups of females, while others remain on the edge of the colony and try to gain matings using alternative strategies (Anderson et al. 1975; Boness & James 1979; Twiss et al. 1994). There is evidence for male philopatry and site fidelity (Twiss et al. 1994; Pomeroy et al. 2000a), which could cause natally site faithful females to be at risk from inbreeding. However, in a mate-defence system such as this one, females will benefit from better resources more than males and are therefore more likely to be natally site faithful (Greenwood 1980). This has been observed in other pinniped species where females are more philopatric than males (Baker et al. 1995; Härkönen & Harding 2001). In addition, females regularly mate with males who do not defend areas within the breeding colony (Worthington Wilmer et al. 1999) and may use other strategies to avoid inbreeding (Amos et al. 2001b). Therefore, it seems unlikely that this is an important factor in reducing natal site fidelity.

While genetic structuring appeared absent in most of the colony there was evidence of kin clustering in Fianuis South (Table 3.4). This may be due to regional differences in

habitat. Mothers in Fianuis South typically cluster into a few main areas (Figure 3.2) that allow good access to water. Having higher natal site fidelity in this region may be more beneficial than in the other two regions where habitat quality and access to water is more consistent. For instance, pupping within 100 m of the natal site in the Study Area may still provide reasonable habitat while doing the same in Fianuis South may be much more detrimental. Density is also lower in Fianuis South, although it can be comparable to other regions around the main areas of use (Figure 3.2). This could lead to less competition in this region, which may allow females to pup near their natal site. Furthermore, Fianuis South was the only region where average female relatedness was significantly greater than zero, suggesting that females may tend to be more natally site faithful to this region as a whole (Figure 3.5). Unfortunately, only one year of data from Fianuis South was available with a small sample size (n = 45 mothers). To confirm that kin clustering regularly occurs in this region, data from additional years are needed.

Overall, results indicate a lack of temporal kin clustering of mothers. For all regions and years, there was no direct relationship between the relatedness of pairs and the time between pupping dates (Table 3.4). However, there was some evidence suggesting that related females tended to pup at similar times (Table 3.5). This pattern was seen in Fianuis South in one year and the Study Area for two years while the opposite trend occurred in Fianuis North. These patterns did not appear consistent though and were not significant after correcting for multiple comparisons.

Individuals may benefit from breeding at the same time within the season as their parents since they were born then and survived to breed, but only if conditions are constant over years. Weather conditions do vary between years but the overall timing and duration of the breeding season on North Rona is generally consistent. However, physiological differences in females may also be important in individual pupping date variation. In the UK, females are mated on the breeding colony in the autumn but implantation of the embryo is delayed until the spring (Boyd 1984). While environmental factors such as daylight hours (Boyd 1991) and sea surface temperature (Coulson 1981) are important in determining an implantation date for all females within an area, body composition may affect individual timing (Boyd 1984). Furthermore, older, higher quality females tend to pup earlier, suggesting pupping date is determined by individual quality (Anderson & Fedak 1987; Pomeroy et al. 1999). Pupping date

may also depend on a female's history, as those that do not breed in one year return to pup earlier in the subsequent year (Pomeroy et al. 1999). Hence, if pupping date is determined mainly by individual quality and previous history, females may not pup at the same time as their kin due to differences in condition.

With the exception of those in Fianuis South, neighbouring mothers that were considered likely to interact (on a spatial and temporal basis) were no more related to each other than expected (Figure 3.6 and 3.7). Even in the absence of natal site and temporal fidelity, individuals may seek out kin if they gain fitness benefits from interacting with them (Baglione et al. 2003). Specifically, grey seal mothers may benefit from aggression reduction between kin (Chapter 5). However, the lack of fine-scale kin clustering found here suggests that pupping near kin may either not be possible, due to competition with conspecifics, or may not be advantageous. Social behaviours based on kinship may be unable to evolve in this population simply because close relatives do not interact on a regular basis.

Overall, samples were representative of mothers pupping within each region with a few exceptions (Figure 3.2; 3.3; Table 3.2). Specifically, certain locations within the Study Area were rarely sampled due the high level of disturbance this would have caused. Therefore, the behaviour of mothers in these locations could not be taken into account. Although sampling tended to be consistent, samples in certain years were sometimes biased towards specific times or places. There is a possibility that this may obscure patterns of genetic structure or produce spurious results. However, this is unlikely, especially for the Study Area, as no significant results were found in any year regardless of the type of sampling bias.

In summary, there was no evidence for genetic structuring between regions in the North Rona colony. This was probably due to both gene flow and a short generation time since the colony was founded. A lack of fine-scale kin clustering of mothers within regions suggests low female natal site and temporal fidelity. Changing habitat quality, competition for preferred sites, and differences in female quality may drive individual pupping site and date selection in the colony. Kin clustering in Fianuis South may be a result of dispersed areas of good habitat increasing the benefit of natal site fidelity and lower female density reducing competition for sites.

Chapter 4: Fine-scale structuring of genetic diversity and relatedness in relation to breeding habitat quality of female grey seals, *Halichoerus grypus*

4.1 Introduction

The habitats that animals use to live and reproduce vary in quality at different spatial and temporal scales (Andrén 1990; Petit & Petit 1996; Danchin et al. 1998). Individuals may use physical cues, or the presence and performance of conspecifics, to assess habitat suitability (Lima & Zollner 1996; Danchin et al. 1998; Brown et al. 2000). If variation in breeding habitat affects reproductive success, and good habitat is limited, competition for sites may occur. This can result in members of a population with lower competitive ability reproducing in sub optimal habitat where they may experience lower fitness (Andrén et al. 1990; Holmes et al. 1996; Rednón et al. 2001). Conversely, higher quality individuals may breed in the best habitats (Petit & Petit 1996; Silverin 1998; Rednón et al. 2001) and invest more in defending them from conspecifics (Silverin 1998; Nijman & Huets 2000; Gray et al. 2002). This is likely to have consequences for the distribution of genetic diversity and relatedness within a population.

Mating between close relatives can reduce the fitness of offspring, a phenomenon known as inbreeding depression. This arises because of the effects of overdominance and deleterious recessive alleles (Charlesworth & Charlesworth 1999). Genetic diversity estimates, derived from marker loci, can represent genome-wide heterozygosity and thus be correlated to the inbreeding coefficient (Hedrick et al. 2001; Hansson & Westerberg 2002). Indeed, genetic diversity of molecular markers, such as microsatellites and allozymes, has been positively correlated with fitness traits in many studies of laboratory and wild populations (reviewed in Britten 1996; Hansson & Westerberg 2002; Keller & Waller 2002; Coltman & Slate 2003). However, recent studies suggest that genetic diversity measures may not be correlated to the inbreeding coefficient and correlations that do exist are likely to be weak, especially for populations that do not experience high variance in levels of inbreeding (Hansson et al. 2001; Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004). In these situations, associations between genetic diversity and fitness traits are more likely to be the result of selection on loci that are linked to markers (Bierne et al. 1998; Hansson et al. 2001; Hansson & Westerberg 2002; Slate et al. 2004).

One effect of low genetic diversity can be to reduce the competitive ability of individuals (Höglund et al. 2002; Tiira et al. 2003; Hoffman et al. 2004). Thus, those members of a population with low genetic diversity may be unable to successfully compete for access to the best sites when there is variation in habitat quality within a population's range. Individuals with low genetic diversity would therefore be expected to consistently inhabit poorer quality habitats, especially when competition is fierce. In support of this, Höglund et al. (2002) found that wild male black grouse (*Tetrao tetrix*) with low genetic diversity were less likely to gain territories or hold them in central locations on a lek.

Another consideration is that there may be spatial and temporal variation with respect to the number of relatives individuals have in the population. Those breeding in better habitat may be more likely to reproduce successfully than those in marginal sites, leading to reproductive skew (Holmes et al. 1996; Silverin 1998; Rednón et al. 2001). Differential behaviour towards kin can help individuals with close relatives in the population to obtain territories, which can increase their reproductive success (Piertney et al. 1999). Breeding site fidelity and the inheritance of territories can also reinforce spatial patterns of relatedness by creating successful lineages that breed in quality habitat.

The grey seal breeding colony on North Rona has several characteristics that make it an ideal population to examine fine-scale spatial and temporal patterns of genetic diversity and relatedness. First, there is evidence of a positive association between genetic diversity and reproductive success of both males and females (Amos et al. 2001a), suggesting genetic diversity could also affect competitive ability. Second, although kin clustering was not detected in the colony (Chapter 3), Pomeroy et al. (2001) found that breeding females that were more related than average to the colony as whole were clustered together. These clusters formed in central areas of the colony where mothers had pups with higher growth rates.

Several other features of the breeding colony suggest that fine-scale spatial patterns of both genetic diversity and average colony relatedness may be present. Breeding behaviour of grey seals has been found to be moderately polygynous, by both behavioural (Anderson et al. 1975; Boness & James 1979; Twiss et al. 1998) and

genetic (Amos et al. 1993; Worthington Wilmer et al. 1999) data. Females also display reproductive skew within the colony (Pomeroy et al. 1999). Furthermore, females may be reproductively active for at least 25 years (Pomeroy et al. 1999) and males for 15 years (Worthington Wilmer et al. 1999). Both sexes tend to be philopatric (Allen et al. 1995; Pomeroy et al. 2000a) and show interannual site fidelity to previous breeding sites within the colony (Twiss et al. 1994; Pomeroy et al. 1994; in press; Chapter 3). Some females also return to pup near the site within the colony where they were born (Pomeroy et al. 2000a).

Grey seals in the UK typically pup on remote coasts or offshore islands, using a variety of habitat types. In some colonies, especially those consisting entirely of sandy beaches (e.g. the Monach Isles) there is little variation in habitat. However, in many colonies on rocky islands or coasts, including North Rona, habitat quality varies substantially over the breeding colony. Availability of water is thought to be the most important feature in pupping site selection as mothers preferentially occupy sites with easy access to water (Pomeroy et al. 2000b; Twiss et al. 2001). Lactating females in the UK may suffer from thermal stress (Twiss et al. 2002) and use the pools for drinking and bathing. When water is scarce, mothers spend less time with their pups, which may lessen pup provisioning or lead to mother-pup separation (Twiss et al. 2000a; Redman et al. 2001).

The distribution of suitable habitat within a colony can lead to variation in the density of breeding females, with clusters forming near access points and pools of water (Anderson et al. 1975; Pomeroy et al. 2000b; Twiss et al. 2001; 2002). Negative density-dependent effects have been reported for other pinniped species where crowding can be intense (Reiter et al. 1981; Harcourt 1992; Baldi et al. 1996). It has been suggested that high female densities may also be detrimental to the survival of grey seal pups due to greater disturbance by conspecifics (Boyd et al. 1962; Summers et al. 1975). Conversely, females in lower density areas may experience more harassment by males (Boness et al. 1995). However, recent studies have failed to detect a correlation between local density and pup survival (Pomeroy et al. 2000b; Twiss et al. 2003).

Habitat quality and female density also vary over the breeding season, which can last for several months (Anderson et al. 1975; Summers et al. 1975; Redman et al. 2001;

Twiss et al. 2002). Since the average duration of lactation for grey seals on North Rona is only 17 days (Pomeroy et al. 1999), there is a turnover of mothers during the season. On North Rona, conditions tend to get cooler and wetter as the season progresses (Anderson et al. 1975; Twiss et al. 2002). However, mothers who pup early have access to the best habitats (Pomeroy et al. 2000b; Twiss et al. 2000a) and are typically larger and have faster growing pups (Anderson & Fedak 1987; Pomeroy et al. 1999). Although mothers have relatively consistent interannual pupping dates, older mothers tend to pup earlier (Pomeroy et al. 1999).

4.2 Aims

The aim of this chapter was to investigate spatial and temporal patterns of genetic diversity and mean relatedness to the colony using mothers breeding in the North Rona grey seal colony. Mothers with similar genetic diversity and mean relatedness to the colony estimates were expected to cluster together in the same locations each year. As low genetic diversity may decrease competitive ability, mothers with lower genetic diversity were predicted to pup in areas of poorer quality habitat (i.e. further from access to water) and later in the season. As density is likely to be low in poor habitat (Anderson et al. 1975; Pomeroy et al. 2000b; Twiss et al. 2001), mothers with lower genetic diversity were also expected to pup in areas of lower density. Mothers with high mean relatedness to the colony were expected to show the same relationship to habitat characteristics and pupping date as found by Pomeroy et al. (2001). That is, they show no relationship to pupping date, ease of access to pools or density but were an optimal distance to access points to the sea. Analyses were performed using data from four breeding seasons to assess annual variation.

4.3 Methods:

4.3.1 Sampling

This chapter used females that were recorded pupping in the colony in 1997, 2000, 2001 and 2002 (as described in Chapter 3). Information on genotyping can be found in Chapter 2. Briefly, each mother was genotyped for between seven and 11 loci. The 1997 data set was supplemented by 96 samples genotyped in Bill Amos's lab in Cambridge. These samples were genotyped for nine of the same loci (not Hl15 and Lc6) and were directly comparable to those genotyped in St. Andrews (Chapter 2). Samples were taken from three regions in 1997: the Study Area, Fianuis South and Fianuis North

(Figure 3.1). Mothers recorded from 2000 to 2002 that were used in this chapter were from the Study Area only, as few samples were taken in the other regions in these years (see Chapter 3). In 1997, pupping locations of mothers were recorded in 10 m^2 grid cells while from 2000 to 2002 pupping locations were recorded to within 2 m accuracy. For spatial autocorrelation analyses, all mothers within a year were given unique locations. Therefore, when two or more mothers had the same pupping location, x and y coordinates were adjusted by 1 m, up to a maximum of 3 m, to ensure unique locations. This adjustment should not significantly affect the results of the analyses at the spatial scales used.

4.3.2 Genetic variables

Standardized heterozygosity (SH) was used as a measure of genetic diversity, as this accounts for the presence of missing data (Coltman et al. 1999) and some individuals were not genotyped at all 11 loci (Chapter 2). SH was calculated by taking the number of heterozygous loci for an individual and dividing by the sum of the observed population heterozygosities of those loci at which the individual was typed (Table 2.4). SH was not normally distributed (see results) and was analysed using non-parametric statistics unless otherwise indicated.

Relatedness between mothers was estimated using RELATEDNESS 5.0 (Queller & Goodnight 1989) using background allele frequencies from 412 adults from the North Rona colony (Chapter 2). For each mother in each year, the relatedness to all other mothers recorded in that year was estimated. The mean relatedness to the colony (MRC) was taken as the mean of these relatedness estimates. Therefore, a mother sampled in two years had two MRC estimates based on the females recorded in that year. Only in 1997 were the MRC estimates calculated using mothers that were recorded in Fianuis South and Fianuis North as well as the Study Area. The analyses performed by Pomeroy et al. (2001) also used mothers from all three pupping regions, sampled in the 1996 breeding season. Therefore, results from 1997 will be the most comparable with those from Pomeroy et al. (2001). MRC was normally distributed (see results) and was analysed using parametric statistics throughout.

4.3.3 Measures of spatial structure

Spatial autocorrelation occurs if values of a variable at one locality are dependent on the values at neighbouring localities (Sokal & Oden 1978). SH and MRC were tested for global spatial autocorrelation using the Moran's I coefficient, which measures the degree of similarity of neighbouring values within specified proximities (lag distances) over the entire area of study. Positive results indicate that similar values are spatially clustered while negative results indicate that spatially clustered values tend to be different. Results close to zero suggest values are distributed at random. To assess at what scale, if any, spatial autocorrelation of SH and MRC occurred in the North Rona breeding colony, Moran's I was calculated at cumulative distance intervals of 10 m up to 50 m (i.e. 0 - 10 m, 0 - 20 m, 0 – 30 m, 0 - 40 m, 0 – 50 m) for each year separately. The lower values of 10 m and 20 m represent scales at which mothers may interact with each other (Pomeroy et al. in press). The upper limit of 50 m is approximately a quarter of the width of the Study Area (Figure 3.1). A Monte Carlo approach was used to test for significant spatial autocorrelation using 10 000 simulations.

The use of global spatial autocorrelation statistics is limited in that it assumes the pattern or process of interest is stable over space, which is often unrealistic (Unwin 1996). For instance, if spatial autocorrelation is positive in some regions but negative in others, the global statistic may be close to zero. Additionally, they indicate only whether there is a pattern over the entire sample but not how it is distributed. Therefore, global spatial autocorrelation would not reveal where there were areas of high and low SH or MRC in the colony. In order to examine clustering of high and low SH and MRC, the local spatial autocorrelation (LSA) statistic Gi* was used (Getis & Ord 1992; Ord & Getis 1995). Tests using LSA statistics in the presence of global spatial autocorrelation have been found to be unreliable, although they are still useful as exploratory tools (Ord & Getis 1995; Sokal et al. 1998). Significant global spatial autocorrelation was only found for one year, using SH (see results), and thus most LSA measures will not be affected. Additionally, an unbiased method for estimating appropriate p-values for LSA is still being developed (Leung et al. 2003). Thus, LSA was used only to indicate where clusters of mothers with high and low SH and MRC may occur and the significance of Gi* values was not tested.

The Gi* measure compares the value at an individual location, and all the values within a given radius around that location, to the global mean (Getis & Ord 1992). Therefore, this approach provides indices of spatial autocorrelation for each sample location. A high Gi* value indicates a clustering of mothers with high SH or MRC with respect to the global mean. Conversely, a low Gi* value indicates a clustering of mothers with low SH or MRC with respect to the global mean. This differs from the Moran's I statistics where a low estimate suggests values are more different from each other than at random.

Three radii were used for LSA analyses: 10 m, 30 m and 50 m. The 10 m radius represents a scale that is relevant to mothers in terms of their social interactions with other females (Pomeroy et al. in press). The 30 m radius was used since this was the distance at which the greatest global spatial autocorrelation was observed (see results). Recent estimates of female site fidelity have also been close to 30 m (Redman 2002; Chapter 3), and hence this is the approximate scale at which patterns may be expected to persist. Finally, a radius of 50 m was used as global spatial autocorrelation was also observed at this scale (see results). Furthermore, this is the scale at which males are site faithful (Twiss et al. 1994). A study of branded females on North Rona also estimated female site fidelity at close to 50 m (Pomeroy et al. 1994). All spatial autocorrelation statistics were calculated using Rookscase Visual Basic routine (Sawada 1999).

Autocorrelation analyses were performed separately for all years and in 1997 the Study Area was analysed separately to allow direct comparisons with the other years. After calculation of the Gi* statistic for each radius, samples with no neighbours were removed for subsequent analyses. To identify areas where mothers had higher and lower than average SH and MRC, standardized normal values (ZGi*) were divided into high, medium, and low categories using +/- 1 standard deviation (SD) of the mean as a cut off. This was considered a useful cut off as high and low categories included only those samples that occurred on the ends of the distribution while categories also contained a sufficient number of samples to allow patterns to be visualised. High and low categories should each contain approximately 16% of the sampled locations as ZGi* follows a standardized normal distribution. Each sample location was plotted onto a map of the colony with the corresponding ZGi* category indicated. In general, high and low ZGi* values will tend to occur in the same locations since they are not

independent (i.e. most of the same mothers will be used to estimate LSA for neighbouring samples). Therefore, this method will usually create groups of high and low ZGi* values within the area of study. To determine whether groups formed in the same location in each year, plots were compared visually. As this technique is likely to be subjective (Unwin 1996), the similarity of ZGi* values within the Study Area were compared statistically between years. The mean of the ZGi* values in each 10 m^2 grid cell was determined. The mean ZGi* values were then compared for each 10 m^2 grid cell that was sampled in both years, for all year combinations, using Pearson's correlations.

It was also important to determine whether groups of mothers with high and low ZGi* values actually represented differences in SH and MRC. The SH of mothers in high (+1 SD) and low (-1 SD) ZGi* categories were compared to those in the medium category using Mann-Whitney U tests. This was repeated for MRC using t-tests. A high or low ZGi* value at an individual location does not necessarily indicate that the mother at that location has a particularly high or low SH or MRC value but rather that mothers within a given radius are generally higher or lower than average. However, these mothers are at the centre of high and low ZGi* groups and should therefore provide an appropriate sample to test whether patterns of ZGi* values represent actual differences in genetic measures. Also, these cut offs have previously been used to examine differences in maternal performance and pupping date (Pomeroy et al. 2001), and it is therefore interesting to explore whether these mothers actually differ in either genetic measure.

4.3.4 Temporal patterns

The pupping date of each mother was recorded directly or estimated from the stage of the pup (as described in Chapter 3). Correlations were performed between pupping date and ZGi* values for SH and MRC to test whether clusters of mothers with high SH and MRC consistently formed earlier in the season. Correlations were then performed between SH and MRC directly to investigate whether mothers with higher SH and MRC pupped earlier in the season. Each year was treated separately. The peak pupping date for females in Fianuis North is earlier than those in Fianuis South and the Study Area (Summer et al. 1975; Pomeroy et al. 1994; Table 3.4). Therefore, mothers from Fianuis North were analysed separately.

4.3.5 Pupping site characteristics

Topographic characteristics of locations of sampled mothers were determined by S. Twiss using a sub-meter resolution digital terrain model (DTM) in an ARC-INFO GIS, created using aerial photographs of the North Rona colony taken in 1994 (Twiss et al. 2000b). From this DTM, grids describing cost-distance to pools and cost-distance to access to the sea were generated. Cost-distance indices model the relative ease of moving over a terrain from one location to another. The route modelled is to the 'nearest' pool or access point respectively, where 'nearest' means the target that can be reached with the least cost. The route is dictated by the nature of the terrain traversed, such that sheer cliffs are avoided and steep inclines increase the cost of movement. Thus, each location receives a cost-distance value representing the relative ease of access to the 'nearest' pool or access point from that location (see Twiss et al. 2000a). The original grids stored topographic data in 0.2 m grid cells. This analysis was conducted at a 10 m grid cell resolution. The original grids were aggregated to form 10 m grid cells where the value of each 10 m grid cell was the median of its 2500 constituent 0.2 m sub cells. Since the locations of pools change throughout the season, grids of cost-distance to pools were generated for five different dates. The cost-distance value from the date closest to the pupping date of each mother was used. No topographic characteristics were available for Fianuis North and for two mothers in Fianuis South and thus these mothers (n = 96) could not be included in the analyses of habitat characteristics.

Locations of all females in the Study Area and most of Fianuis South were mapped daily throughout the field season each year (by P. Pomeroy). These locations were entered into the GIS and used to estimate density. Local density was taken as the number of females within a 10 m radius of a mother's pupping location on the date she gave birth. This scale was used to obtain a local measure of density that is relevant to mothers; it is also at the same scale as the topographic data. For mothers with pupping dates before the start of mapping, no density estimates were recorded (1997 n = 3; 2000 n = 3; 2001 n = 4; 2002 n = 0). However, density was always low before the start of mapping and thus these females were assigned a density estimate of one. As with the topographic data, density estimates were not available for Fianuis North and for two mothers in Fianuis South (n = 96).

Four sets of multiple linear regression models were created with the following response variables: ZGi* for SH, ZGi* for MRC, SH and MRC. The ZGi* models were used to assess whether clustering of values consistently correlated to particular habitat characteristics. For example, mothers with high ZGi* values (i.e. mothers at the centre of clusters of high SH or MRC) may occur close to pools every year. Models using SH and MRC as response variables will indicate whether there is a direct relationship between characteristics of pupping sites and either genetic variable. The explanatory variables in each model were cost-distance to access, cost-distance to pools and female density. Models were reduced using a backwards stepwise approach, with variables being removed at $p > 0.10$. As cost-distance to access is thought to exhibit a non-linear relationship with habitat quality (Pomeroy et al. 2001; Twiss et al. 2003) the effectiveness of quadratic models to explain variation was also investigated. Non-linear variables were only included in multiple linear regression models if, when tested alone, the relationship was significant and inclusion in the model increased the adjusted r^2 value. The linear cost-distance to access term was always retained in the model if a quadratic was present. All years were analysed separately since datasets were not independent with many mothers present in more than one year. SH was not normally distributed (due to left skew) and thus should not usually be analysed using parametric statistics. However, analyses were found to conform to two assumptions of linear regression models: and homogeneity of variance (Grafen & Hails 2002). Normality of error was checked by testing the residuals for normality, using Kolmogorov-Smirnov goodness-of-fit tests. To test for homogeneity of variance, standardized residuals were plotted against fitted values. These were visually inspected to ensure there was an even scatter of residuals across the range of fitted values. Statistical analyses were performed in SPSS 11.0.

4.4 Results

4.4.1 Characteristics of genetic variables

For all sampled mothers, SH ranged from 0.57 - 1.55 (median = 1.14) and was left skewed (Figure 4.1). Median SH values were similar in each year (Table 4.1) and SH was not normally distributed in any year (Kolmogorov-Smirnov goodness-of-fit test, $p < 0.05$ in all years). The SH of individual mothers was significantly negatively correlated to their MRC in all years (Table 4.1).

Table 4.1: Average standardized heterozygosity (SH) and mean relatedness to the colony (MRC) estimates for each year and results of Spearman's correlations between SH and MRC.

Year	n	SH	MRC	r_s	p
		Median (IQR)	Mean ± SD		
1997	245	1.16 (0.97 – 1.19)	0.001 ± 0.037	-0.409	< 0.001
2000	73	1.05 (1.05 – 1.19)	-0.007 ± 0.039	-0.458	< 0.001
2001	136	1.12 (1.05 – 1.19)	-0.005 ± 0.037	-0.396	< 0.001
2002	121	1.05 (1.05 – 1.19)	-0.005 ± 0.039	-0.426	< 0.001

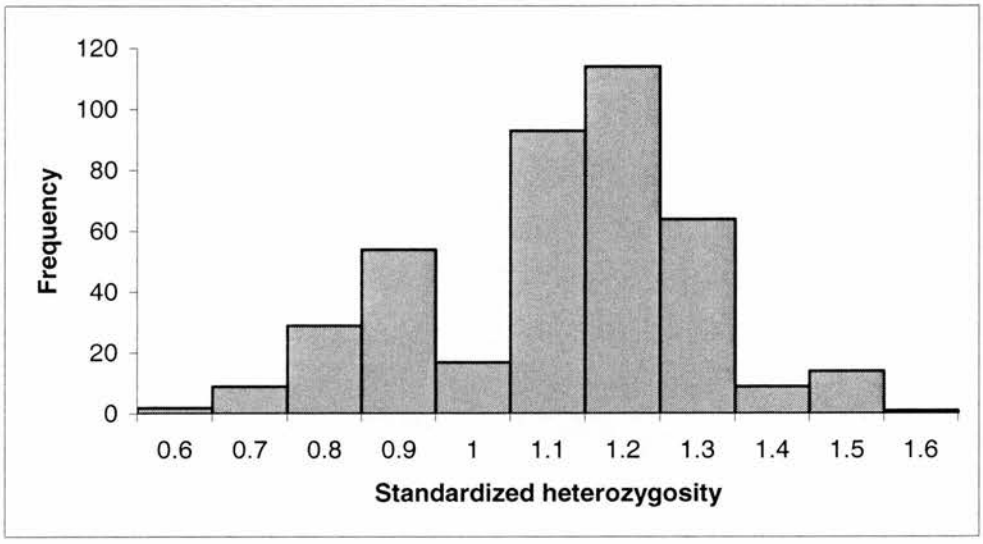


Figure 4.1: Histogram of standardized heterozygosity for all sampled mothers from 1997, 2000, 2001 and 2002 (n = 405).

Mean MRC in each year was close to zero (Table 4.1) and was normally distributed (Kolmogorov-Smirnov goodness-of-fit test, $p > 0.80$ in all years). MRC was estimated separately for each year a mother was recorded, based on the mothers that were sampled in that year. For mothers that were present in more than one year, MRC values were strongly and significantly positively correlated (Table 4.2). Therefore females were consistent in their MRC values, despite different individuals being sampled in the years tested. This strong positive correlation remained even when MRC estimates included mothers from Fianuis North and South in 1997 (Table 4.2). There was no correlation between the number of mothers sampled in both years and their correlation coefficient ($r_s = 0.184$, $n = 10$, $p = 0.611$).

Table 4.2: Pearson's correlations (below diagonal) of mean relatedness to the colony (MRC) between years for females sampled in both years (numbers of females above the diagonal). In 1997 comparisons were made between MRC estimated using females from all regions (1997 (all)) and between MRC estimated using only females from the Study Area (1997 (SA)). For all other years, samples were from the Study Area only. All correlations were significant at $p < 0.001$.

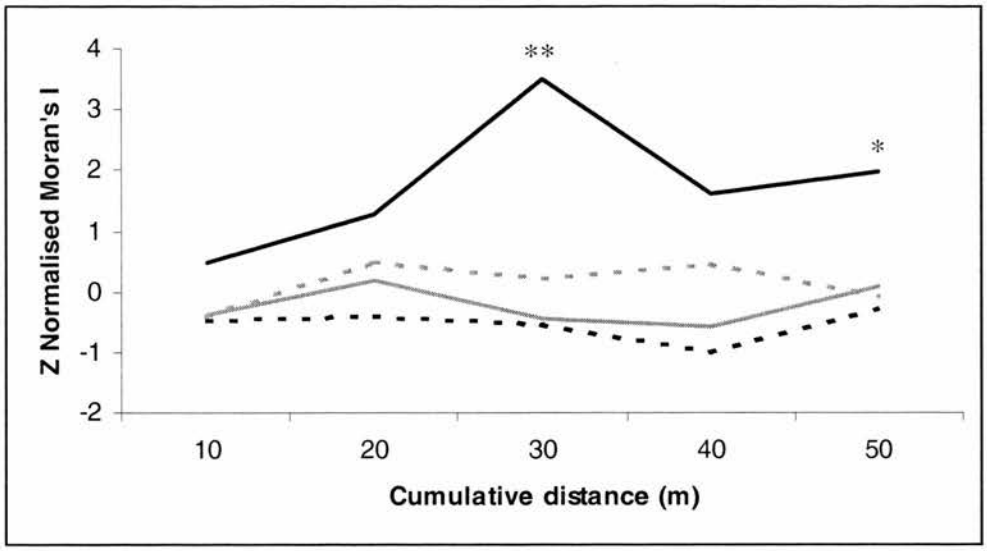
	1997 (all)	**1997 (SA)**	**2000**	**2001**	**2002**
1997 (all)	-----	106	23	33	30
1997 (SA)	0.930	-----	23	33	30
2000	0.912	0.871	-----	42	42
2001	0.864	0.901	0.843	-----	69
2002	0.927	0.916	0.897	0.921	-----

4.4.2 Spatial autocorrelation analyses

There was significant positive global spatial autocorrelation of SH in 1997 at a scale of 0 – 30 m and 0 – 50 m (Figure 4.2A) indicating mothers pupping with 30 m and 50 m had similar SH values. No spatial autocorrelation of SH was apparent in any other year, and no consistent patterns occurred across years (Figure 4.2A). There was also significant but weak positive spatial autocorrelation of SH in 1997 at the 0 – 30 m scale when only the Study Area was examined ($Z = 1.84$, $n = 106$, $p = 0.046$). This suggests the difference between this year and others was not only due to the wider spatial distribution of samples used in 1997 (i.e. inclusion of samples from Fianuis North and South). However, at the 0 – 50 m scale, significant positive spatial autocorrelation of SH was not detected in 1997 when only samples from the Study Area were included ($Z = 0.30$, $n = 106$, $p = 0.326$). This may also be due to the smaller sample size used (106 vs. 245).

No significant global spatial autocorrelation of MRC was found in any year (Figure 4.2B). In 1997, as with SH, the greatest global spatial autocorrelation of MRC was at the 0 – 30 m scale (Figure 4.2B). Over all years, spatial autocorrelation of MRC appeared greater at the 0 – 30 m and 0 – 50 m scale and lower at the 0 – 40 m scale (Figure 4.2B).

A



B

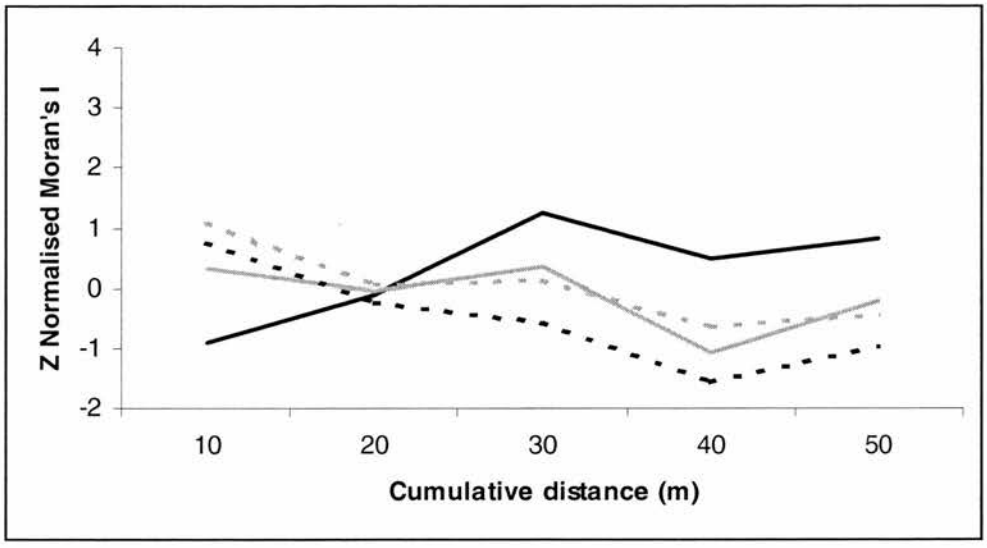


Figure 4.2. Correlograms of Moran's I values for A) standardized heterozygosity and B) mean relatedness to the colony for 1997 (solid black), 2000 (solid grey), 2001 (dashed black), 2002 (dashed grey). * $p < 0.05$, ** $p < 0.01$.

LSA analyses were performed at 10m, 30m and 50m scales. However, the 10 m scale was found to be too local to detect patterns across the colony. This was because many mothers had only a few neighbours that were sampled within a 10 m radius, which were then compared in the Gi* analysis. The percentage of mothers with two or less neighbours for each year was: 1997 47%; 2000 48%; 2001 38%; 2002 34%. Conversely, the 50 m scale was found to be too large to create patterns within the colony. Within a 50 m radius there was a high degree of overlap between samples that were included in Gi* estimates for each mother. This tended to make all Gi* values similar as many of the same SH or MRC values were compared for each mother. Thus,

for the remainder of the chapter only results from the LSA analyses using a 30 m radius will be discussed. The number of mothers with no neighbours at 30 m that were removed for all subsequent analyses was: 1997 n = 6, 2000 to 2002 n = 1 in each year.

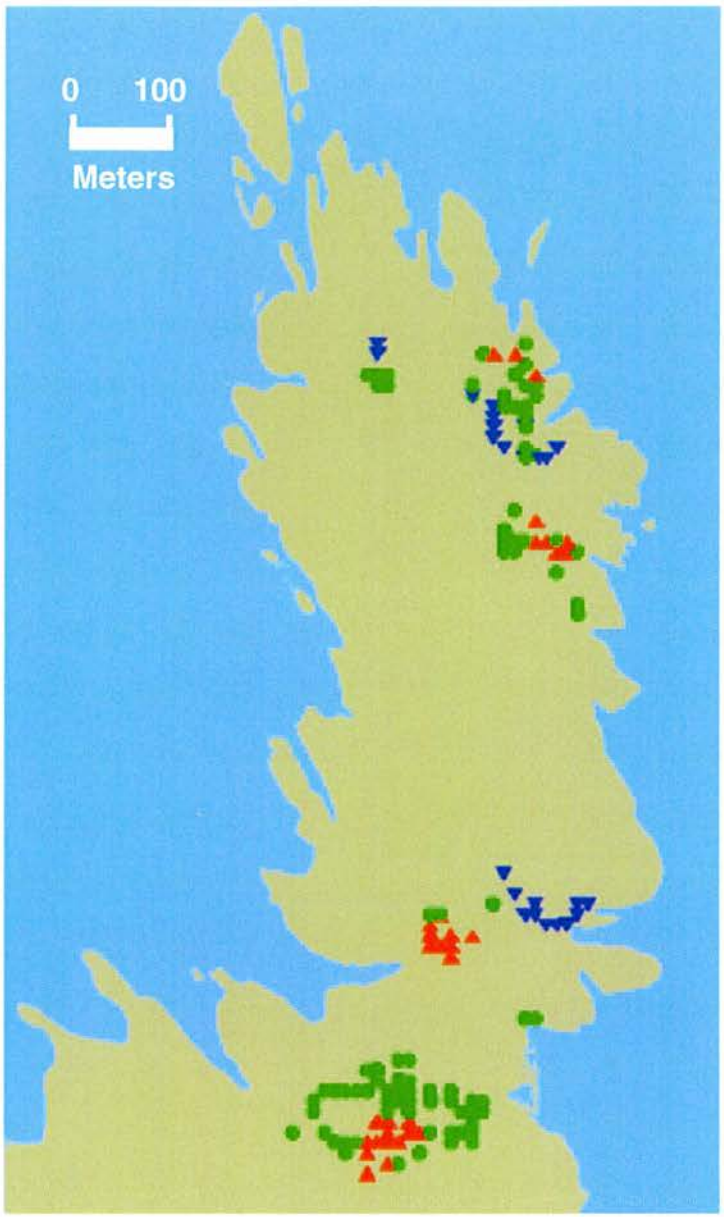
Visual inspection of the LSA results indicated that, as expected, similar ZGi* values tended to group together, creating areas of high and areas of low values (Figure 4.3; 4.4). For the analysis in 1997 of SH, using mothers from all three regions in the Fianuis peninsula, no high ZGi* values (which indicate clustering of mothers with high SH) appeared in the Study Area while groups of low ZGi* values (indicating clustering of mothers with low SH) were present in all three regions (Figure 4.3). Both low and high values tended to form discrete aggregations on the colony, especially in Fianuis South.

As mothers were recorded in different locations in the Study Area in different years a direct comparison between years was difficult to make visually. For analyses using SH, low ZGi* values tended to group on the western side of the Study Area while high ZGi* values tended to group on the eastern side, except in 2001 where the opposite pattern was observed (Figure 4.3). This interpretation was supported by correlations between average ZGi* values in the same 10 m^2 grid cell in different years. Significant positive correlations were found between mean ZGi* values in 1997 and 2000 and in 2000 and 2002 confirming patterns of ZGi* values were similar in those years (Table 4.3). Conversely, ZGi* values were negatively correlated between 2001 and all other years indicating patterns of ZGi* were reversed in this year (Table 4.3). However, only the negative correlation between 2001 and 2002 was significant after Bonferroni corrections for multiple comparisons.

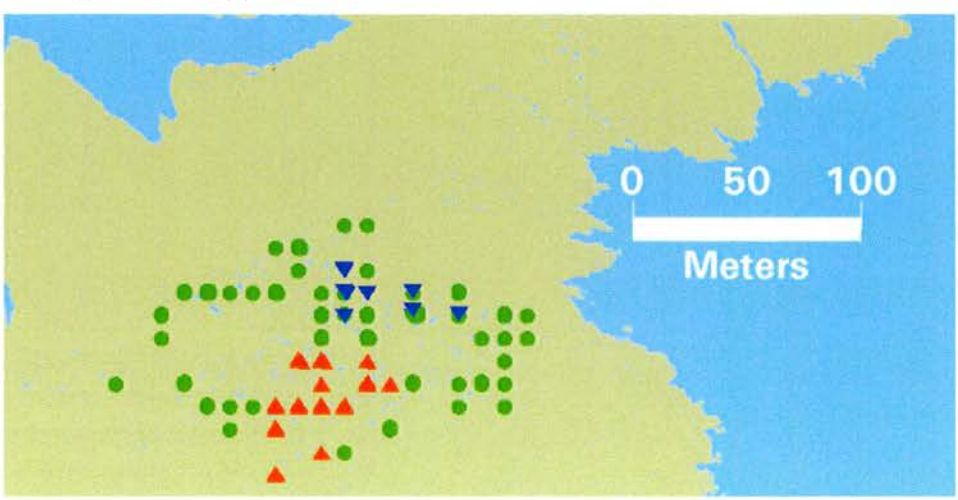
Table 4.3: Pearson's correlations between mean ZGi* values in each occupied 10 m^2 grid cell each year for standardized heterozygosity (below the diagonal) and mean relatedness to the colony (above the diagonal). Numbers in brackets are the number of 10 m^2 grid cells compared. Values on the diagonal are the number of 10 m^2 grid cells that were sampled in each year. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Underlining indicates these correlations were significant after Bonferroni corrections for multiple comparisons.

	1997	**2000**	**2001**	**2002**
1997	**59**	-0.659** (19)	-0.479** (28)	0.685*** (27)
2000	0.505* (19)	**46**	0.266 (27)	-0.445* (24)
2001	-0.379* (28)	-0.429* (27)	**75**	-0.659*** (35)
2002	0.247 (27)	0.490* (24)	-0.663*** (35)	**70**

1997 (All)



1997 (Study Area)



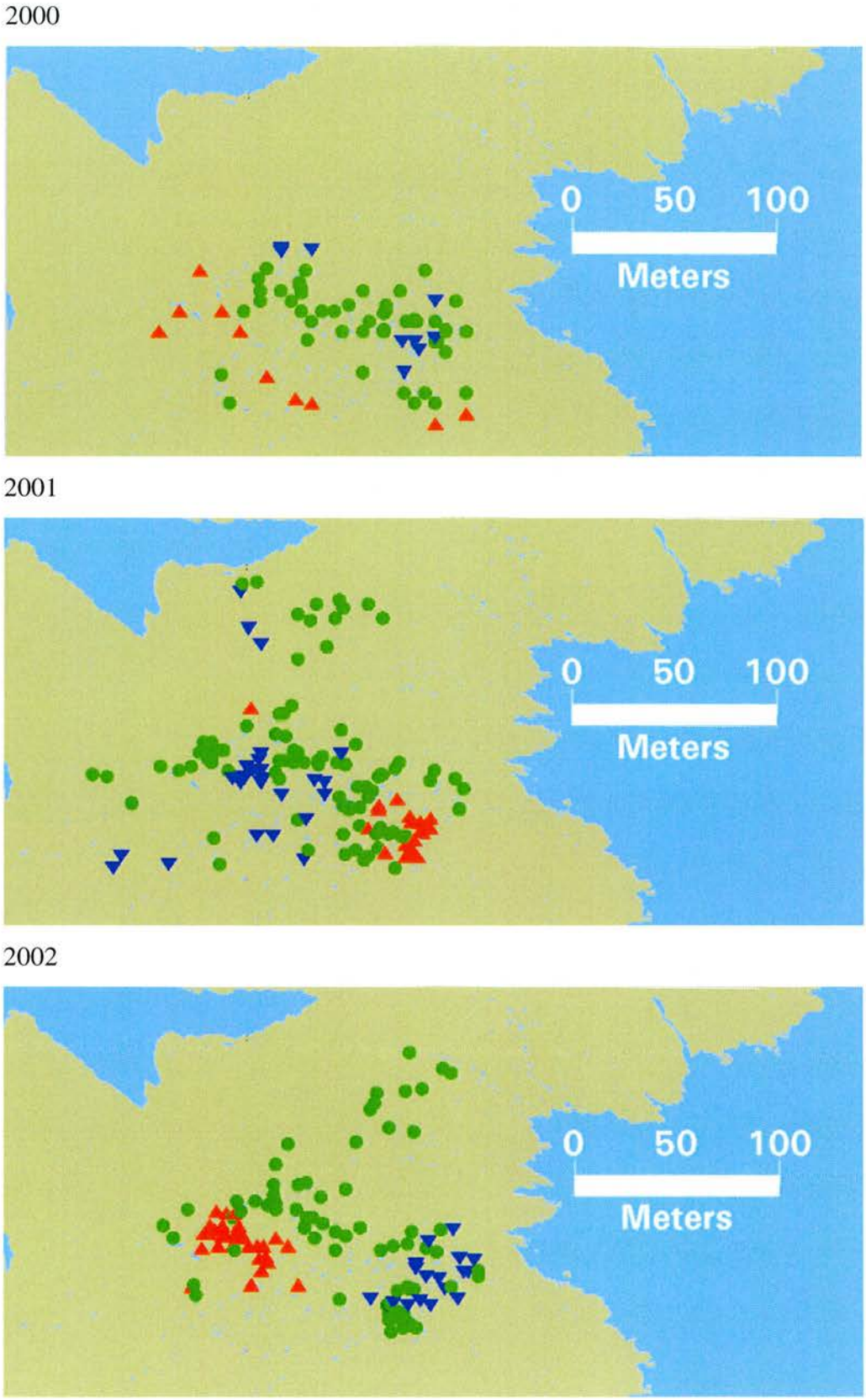
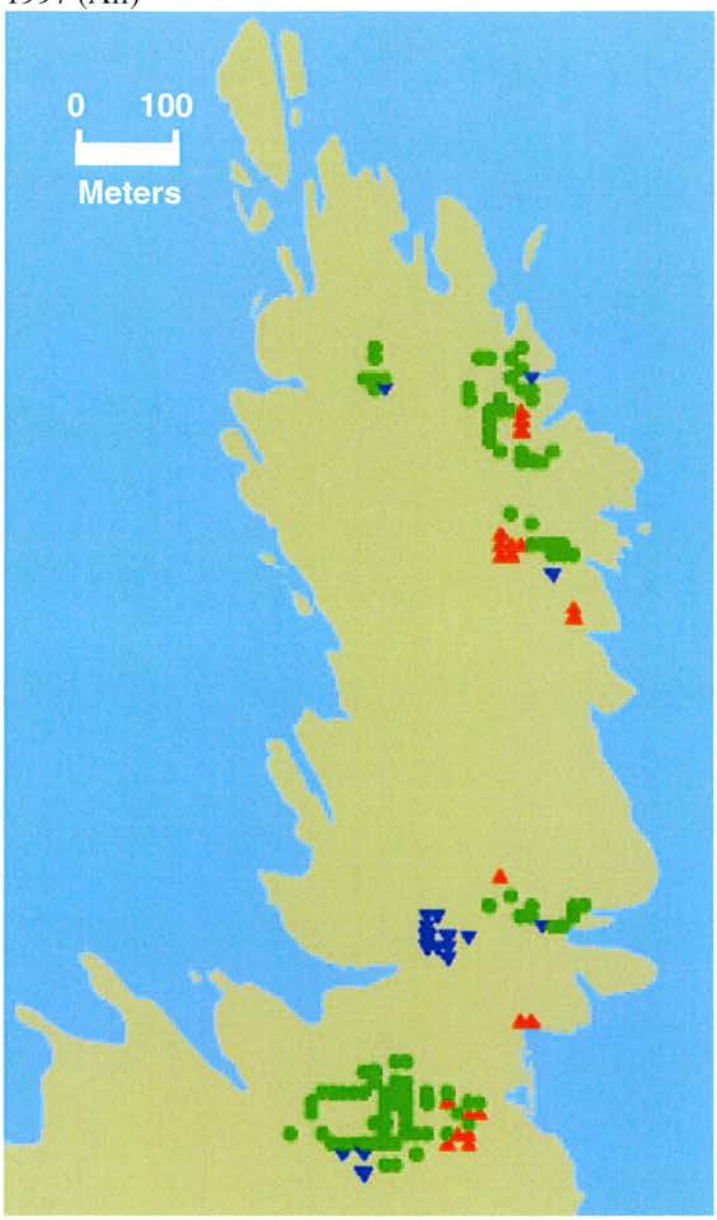
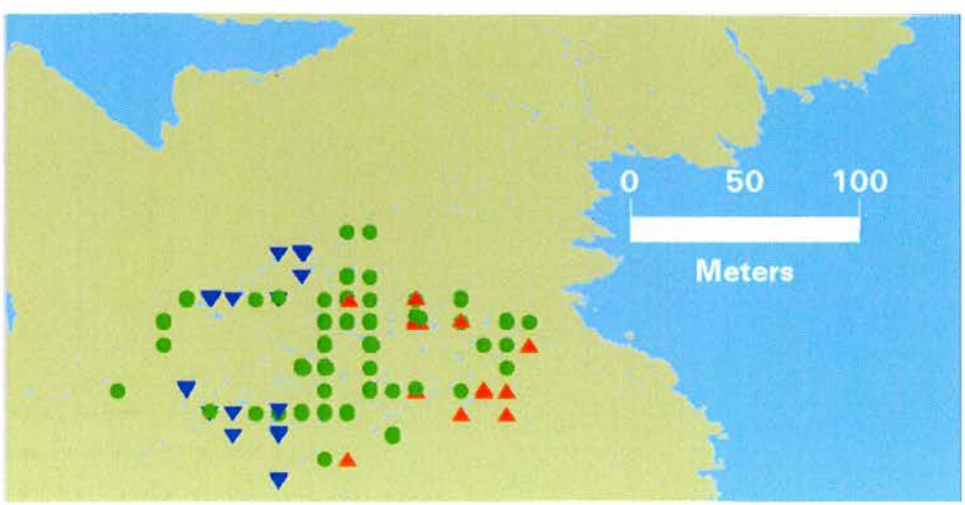


Figure 4.3: The local spatial autocorrelation statistic, ZGi*, for standardized heterozygosity for sampled mothers, displayed on a map of North Rona with land shown in grey and sea and pools of water in blue. Results are for the Study Area only except in 1997 (All) when results from all three pupping regions on the Fianuis peninsula. ZGi* was divided into high (blue triangles), medium (green circles) and low (red triangles) using a +/- 1 SD of the mean as a cut off.

1997 (All)



1997 (Study Area)



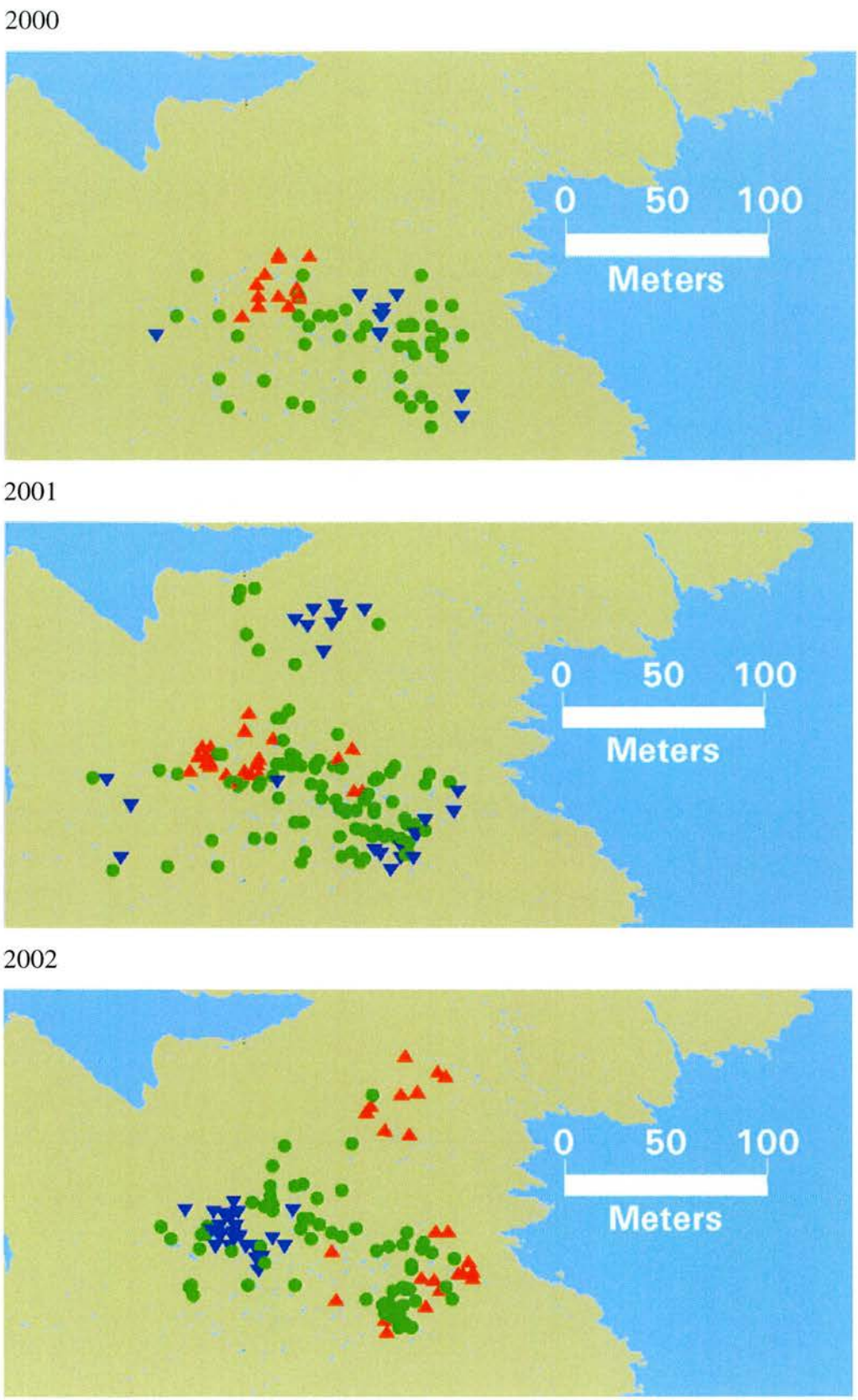


Figure 4.4: The local spatial autocorrelation statistic, ZGi*, for mean relatedness to the colony for sampled mothers, displayed on a map of North Rona with land shown in grey and sea and pools of water in blue. Results are for the Study Area only except in 1997 (All) when results from all three pupping regions on the Fianuis peninsula. ZGi* was divided into high (blue triangles), medium (green circles) and low (red triangles) using a +/- 1 SD of the mean as a cut off.

The LSA for MRC in 1997 using mothers from all three regions in the Fianuis peninsula, showed few high ZGi* values in the Study Area (Figure 4.4). There was also little grouping of values in Fianuis North, with high ZGi* values (indicating clustering of mothers with high MRC) and low ZGi* values (indicating clustering of mothers with low MRC) spread throughout the region. High ZGi* values were most aggregated in Fianuis South.

Within the Study Area, patterns for MRC were similar in 1997 and 2002, with high ZGi* values grouping on the western side and low ZGi* values grouping on the eastern side (Figure 4.4). Conversely, in 2000 and 2001, high values tended to group on the eastern side and low values grouped on the western side (Figure 4.4). Correlations between years of mean ZGi* values within each 10 m^2 grid cell also indicated that patterns of ZGi* values were similar in 1997 and 2002 but not in 2000 and 2001 (Table 4.3). All other correlations were negative, with those between 1997 and 2000 and between 2001 and 2002 remaining significant after Bonferroni corrections for multiple comparisons (Table 4.3).

As SH and MRC were negatively correlated (Table 4.1), patterns of ZGi* were not expected to be similar within years. Indeed, plots of high and low ZGi* values suggest areas of similarly high SH values are not also areas of similarly high MRC values and vice versa (Figure 4.3; 4.4). For example, in Fianuis South in 1997, low ZGi* values for SH were in a similar location to high ZGi* values for MRC (Figure 4.3; 4.4). Correlations within years of mean ZGi* values for SH and MRC in each 10 m^2 grid cell were negative in all years: (1997 $r = -0.128$, $n = 59$, $p = 0.334$; 2000 $r = -0.230$ $n = 46$, $p = 0.125$; 2001 $r = -0.219$, $n = 75$, $p = 0.059$; 2002 $r = -0.679$, $n = 70$, $p < 0.001$). However, this relationship was only significant in 2002; this is well illustrated by the plots in this year, as high and low ZGi* values appear in opposite locations (Figure 4.3; 4.4).

The SH of mothers in the low ZGi* category was significantly less than the SH of mothers in the medium ZGi* category in all years except 2002 (Table 4.4). However, the SH of mothers in the high ZGi* category was only significantly greater than the SH of mothers in the medium ZGi* category in 1997. While some of these correlations may be the result of multiple testing, it seems likely that there is a genuine trend for the low

ZGi* category to be more representative of a mother's SH than the high ZGi* category. Nevertheless, differences in the medians of SH values were small and medians of two or more categories were often identical within a year (Table 4.4). Moreover, in 2000, mothers in the high ZGi* category had lower median SH than those in the medium category. This suggests that categories do not consistently represent actual differences in the SH of mothers.

Table 4.4: Comparison of mothers in high (+ 1 SD) and low (-1 SD) ZGi* categories to those in the medium category for standardized heterozygosity (SH) (using Mann-Whitney U tests) and mean relatedness to the colony (MRC) (using t-tests).

Year	ZGi* category	SH			MRC		
		n	Median	Z	n	Mean	t
1997	High	32	1.18	2.46*	35	0.028	3.79***
	Med	148	1.17	------	169	-0.001	------
	Low	59	0.98	4.40***	35	-0.023	3.42**
2000	High	11	1.05	0.41	10	0.006	0.932
	Med	51	1.19	------	47	-0.007	------
	Low	10	1.05	2.44*	15	-0.021	1.21
2001	High	27	1.19	0.33	23	0.011	1.98*
	Med	89	1.19	------	89	-0.006	------
	Low	19	1.05	2.13*	23	-0.016	-1.17
2002	High	18	1.05	0.23	25	0.003	1.13
	Med	73	1.05	------	71	-0.007	------
	Low	29	1.05	1.39	24	-0.011	0.45

The MRC of mothers in the low ZGi* category was only significantly lower than those in the medium category in 1997 (Table 4.4). The MRC of mothers in the high ZGi* category was significantly higher than those in the medium category in 1997 and in 2001 (Table 4.4). After Bonferroni corrections for multiple testing only the difference in MRC between mothers in the high and medium category in 1997 remained significant. In all comparisons, the mean of the low and high category was lower and higher respectively than the mean of mothers in the medium category.

4.4.3 Temporal patterns

Characteristics of pupping dates of sampled mothers can be found in Table 3.2. ZGi* values for SH were positively correlated with pupping date in 1997 and 2001 but negatively correlated in 2000 and 2002 (Table 4.5). These results agree with the plots from these years, as the eastern side of the Study Area is usually the first part to be colonised each season. In 2000 and 2002, mothers with high ZGi* values for SH

grouped on the eastern side of the Study Area and pupped earlier, while in 2001 mothers with high ZGi* values grouped on the western side and pupped later (Figure 4.3; Table 4.5). A direct comparison with the 1997 plots could not be made as the analysis included females from Fianuis South. The analysis from 1997 did not included mothers from Fianuis North since females breed earlier in this region of the colony. When mothers from Fianuis North were analysed separately, there was no correlation between ZGi* values for SH and pupping date (r_s = -0.139, n = 94, p = 0.181).

Table 4.5: Pearson's correlations between pupping date and ZGi* values for standardized heterozygosity (SH), ZGi* values for mean relatedness to the colony (MRC), SH (Spearman's correlations) and MRC The analysis for 1997 includes mothers from the Study Area and Fianuis South only. * p < 0.05, **p < 0.001, ***p < 0.001.

Year	ZGi* (SH)	ZGi* (MRC)	SH	MRC
1997	0.206*	-0.285***	0.017	-0.005
2000	-0.313**	-0.310**	0.007	-0.010
2001	0.315***	0.005	0.081	0.057
2002	-0.234*	0.080	-0.045	-0.045

ZGi* values for MRC were only significantly correlated to pupping date in 1997 and 2000 and in both cases were negative (Table 4.5). This suggests that mothers at the centres of clusters of high MRC pupped earlier in the breeding season in these years. There were no significant correlations between pupping date and ZGi* for MRC in 2001 and 2002 (Table 4.5). This is surprising for 2002 since the plots for MRC show clustering of high values on the western side and clustering of low values on the eastern side (Figure 4.4). When mothers from Fianuis North were analysed separately, there was no correlation between ZGi* values for MRC and pupping date (r_s = 0.108, n = 94 p = 0.300)

There was no correlation between SH or MRC and pupping date in any year (Table 4.5). This was also the case for Fianuis North in 1997 (SH r_s = 0.088, n = 94, p = 0.401; MRC r_s = 0.103, p = 0.321).

4.4.4 Pupping site characteristics

There was no correlation between the cost-distance to access and the cost-distance to pools of individual pupping locations in any year. However, the density of females at a mother's pupping site was significantly negatively correlated to cost-distance to access

in three years and to cost-distance to pools in all four years (Table 4.6). Thus locations with a higher cost, in terms of accessing water, had a lower density of females.

Table 4.6 Spearman's correlations between mothers' pupping site density and cost-distance to access to the sea (Access) and pools of water (Pools).

Year	n	Access		Pools	
		r_s	p	r_s	p
1997	149	0.155	0.062	-0.250	0.002
2000	73	-0.242	0.043	-0.395	0.001
2001	136	-0.456	< 0.001	-0.251	0.004
2002	121	-0.327	< 0.001	-0.278	0.002

First, models of habitat characteristics were analysed for ZGi* values for SH and MRC to determine whether they displayed the same patterns in each year. Mothers with higher ZGi* values for SH pupped in lower cost-distance to access sites in all years except 2001, when the reverse pattern was observed (Table 4.7A; Reduced models: 1997 $F_{3,139} = 13.10$, $p < 0.001$, $r^2_{(adj)} = 0.197$; 2000 $F_{2,69} = 10.36$, $p < 0.001$, $r^2_{(adj)} = 0.206$; 2001 $F_{3,131} = 18.47$, $p < 0.001$, $r^2_{(adj)} = 0.280$; 2002 $F_{1,118} = 220.20$, $p < 0.001$, $r^2_{(adj)} = 0.646$). These patterns can also be seen in the plots of ZGi* categories for SH as clusters of high ZGi* values appeared close to access points (on the eastern side of the colony) every year except 2001 when the group of high ZGi* values occurred on the western side (far from access points) (Figure 4.3). Mothers with high ZGi* values also pupped in locations with higher densities in 1997; this trend was seen in 2001 as well (Table 4.7A). No other variables significantly explained variation in ZGi* values for SH after Bonferroni corrections for multiple comparisons.

ZGi* values for MRC were also correlated with cost-distance to access, except in 1997 (Table 4.7A; Reduced models: 1997 $F_{1,141} = 3.27$, $p = 0.073$, $r^2_{(adj)} = 0.015$; 2000 $F_{2,69} = 7.50$, $p = 0.001$, $r^2_{(adj)} = 0.155$; 2001 $F_{2,132} = 9.81$, $p < 0.001$, $r^2_{(adj)} = 0.115$; 2002 $F_{2,117} = 75.77$, $p < 0.001$, $r^2_{(adj)} = 0.555$). In both 2000 and 2001 mothers with higher ZGi* values for MRC pupped in lower cost-distance to access sites. Conversely, mothers with higher ZGi* values for MRC pupped in higher cost-distance to access sites in 2002. Thus, the results for ZGi* values for SH and MRC were similar in 2000 but opposite in 2001 and 2002 (Table 4.7A). This agrees with the patterns seen in the plots of these years (Figures 4.3; 4.4). Mothers with higher ZGi* values for MRC also pupped in sites with lower densities in 2001. This trend also occurred in 1997 and 2000. No other variables significantly explained variation in ZGi* values for MRC after Bonferroni

corrections for multiple comparisons. In no year was the relationship between ZGi* and cost-distance to access significantly improved by fitting a quadratic model.

Table 4.7: Multiple linear regressions of A) ZGi* values for standardized heterozygosity (SH) and mean relatedness to the colony (MRC) and B) for SH and MRC to cost-distance to access (Access), cost-distance to pools (Pools) and density of females at the pupping site (Density). Bold values indicate this variable is from the reduced model. All other values are from the full model.

A

		ZGi* (SH)			ZGi* (MRC)		
		Access	**Pools**	**Density**	**Access**	**Pools**	**Density**
1997	B	**-0.42**	**0.13**	**0.30**	0.04	0.10	**-0.15**
	t	**-5.58**	**1.72**	**3.93**	0.48	1.14	**-1.81**
	p	**<0.001**	**0.087**	**<0.001**	0.628	0.255	**0.073**
2000	B	**-0.40**	**-0.22**	0.14	**-0.40**	0.04	**-0.26**
	t	**-3.74**	**-2.04**	1.22	**-3.56**	0.33	**-2.29**
	p	**<0.001**	**0.045**	0.225	**<0.001**	0.73	**0.025**
2001	B	**0.56**	**0.19**	**0.16**	**-0.38**	0.10	**-0.26**
	t	**7.06**	**2.09**	**1.96**	**-4.24**	1.16	**-2.89**
	p	**<0.001**	**0.038**	**0.052**	**<0.001**	0.250	**0.004**
2002	B	**-0.81**	0.08	0.09	**0.74**	**-0.13**	0.02
	t	**-14.84**	1.34	1.47	**12.12**	**-2.16**	0.22
	p	**<0.001**	0.187	0.145	**<0.001**	**0.032**	0.827

B

		SH			MRC		
		Access	**Pools**	**Density**	**Access**	**Pools**	**Density**
1997	B	**-0.00**	0.05	0.11	0.03	0.04	0.03
	t	**-0.01**	0.61	1.24	0.37	0.45	0.33
	p	**0.991***	0.540	0.181	0.709	0.621	0.739
2000	B	0.02	-0.10	**0.31**	-0.16	**0.22**	0.04
	t	0.14	-0.85	**2.75**	-1.30	**1.88**	0.35
	p	0.890	0.396	**0.008**	0.197	**0.065**	0.731
2001	B	0.09	0.11	0.02	0.00	-0.05	-0.01
	t	0.10	1.28	0.15	0.03	-0.61	-0.06
	p	0.338	0.203	0.880	0.978	0.545	0.995
2002	B	-0.08	-0.02	**0.19**	0.13	-0.05	0.04
	t	-0.78	-0.20	**2.14**	1.29	-0.53	0.36
	p	0.438	0.841	**0.034**	0.198	0.596	0.720

* This variable was best explained by a quadratic term as described in the text.

Second, models of habitat characteristics were analysed using actual values of SH and MRC for each mother. Following stepwise reduction of the SH regression models, the cost-distance to pools was not retained in any year (Table 4.7B). There was a non-significant relationship between the SH of mothers and the cost-distance to access of the pupping site in 1997 which was best explained by a quadratic relationship (Figure 4.5; B = 0.22, t = 1.87, p = 0.063; Reduced model: $F_{2,146} = 3.60$, p = 0.030, $r^2_{(adj)} = 0.034$).

Inclusion of only the linear cost-distance to access term in the regression model resulted in less variation being explained ($r^2_{(adj)} = 0.017$). Nevertheless, the adjusted r^2 values for both models were low. When the relationship between cost-distance to access and SH was plotted, SH decreased with increasing cost-distance to access to a value of approximately 50 and then increased (Figure 4.5). However, the quadratic relationship appeared weak and was associated with a large amount of scatter. In no other year was the relationship between SH and cost-distance to access significantly better explained by a quadratic model; this term was not retained in models in other years (Table 4.7B).

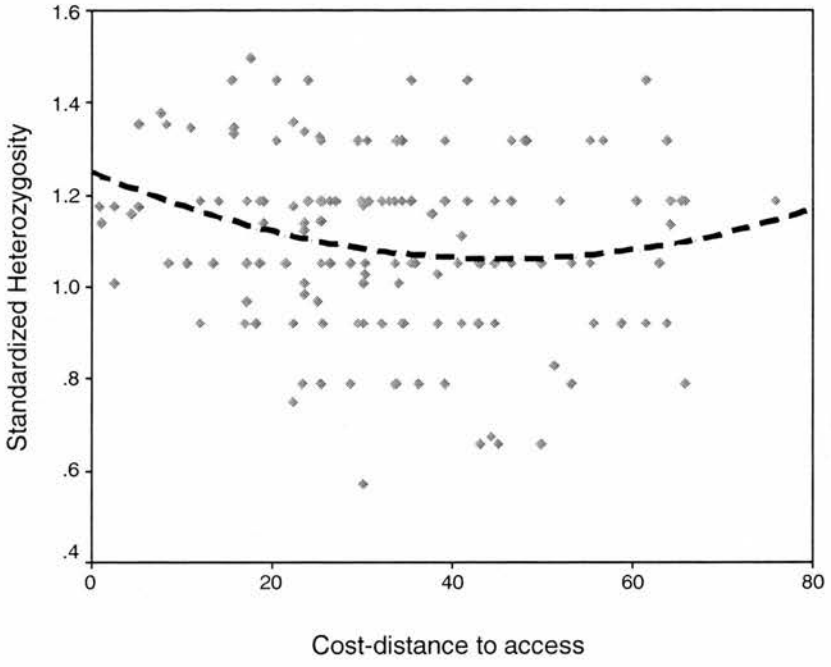


Figure 4.5: Relationship between cost-distance to access of pupping sites and mothers' standardized heterozygosity. This was best explained by the quadratic model $-0.0083x + 0.000091x^2 + 1.2502$.

In 2000 and 2002, mothers with higher SH pupped in areas of higher density (Table 4.7B; Reduced model: 2000, $F_{1,71} = 7.57$, $p = 0.008$, $r^2_{(adj)} = 0.084$; 2002, $F_{1,119} = 4.56$, $p = 0.034$, $r^2_{(adj)} = 0.029$). Only the result in 2000 was significant after Bonferroni corrections for multiple comparisons. In 2001, no habitat characteristic significantly explained any of the variation in mothers' SH (Table 4.7B; Full model: $F_{3,132} = 0.945$, $p = 0.421$, $r^2_{(adj)} = 0.001$).

In 2000, mothers with higher MRC tended to pup in areas with higher cost-distance to pools, but not significantly so (Table 4.7B; Reduced model: $F_{1,71} = 3.52$, $p = 0.065$, $r^2_{(adj)} = 0.034$) Habitat characteristics failed to significantly explain variation in mothers'

MRC in all other years (Table 4.7B; Full models: 1997, $F_{3,145} = 0.19$, $p = 0.904$, $r^2_{(adj)} = -0.017$; 2001, $F_{3,132} = 0.13$, $p = 0.944$, $r^2_{(adj)} = -0.020$; $F_{3,117} = 0.71$, $p = 0.546$, $r^2_{(adj)} = -0.007$). Overall, adjusted r^2 values from models of both SH and MRC were low, with the highest value, attained in the 2000 model of SH, being only 0.085. Thus, even when models reached significance, habitat characteristics explained only a small amount of variation in SH and MRC.

4.5 Discussion

Previous studies have suggested that female grey seals with higher genetic diversity have greater reproductive success (Amos et al. 2001a) and that mothers in higher quality habitats are more successful (Twiss et al. 2000a; Pomeroy et al. 2001). However, this study found no conclusive evidence that genetic diversity of mothers correlated with the quality of their pupping site in the North Rona colony (Table 4.7). Nor did it find evidence to support the result of Pomeroy et al. (2001) that mothers with higher than average relatedness to the colony pup an optimum distance away from access points to the sea (Table 4.7).

In all years, standardized heterozygosity of mothers was negatively correlated to their mean relatedness to the colony (Table 4.1). Relatedness was calculated using the amount of allele sharing between pairs while accounting for the frequency of those alleles in the population (Queller & Goodnight 1989). Mothers with common alleles share them with more individuals in the population and thus will have higher relatedness scores to more females, but since the alleles are common, these mothers are more likely to be homozygotes. In contrast, offspring from immigrants will tend to have high heterozygosity and few relatives in the colony. The original hypotheses were that mothers with high genetic diversity and high relatedness to the colony would pup in preferred habitats. However, both predictions are unlikely to true. If genetic diversity is a good predictor of individual quality and competition for sites is high, this variable may be a better predictor of spatial and temporal patterns. However, if these patterns reflect successful lineages, and patterns are maintained through historical site use, then average relatedness to the colony may be a better predictor of mothers' habitat characteristics.

Of the two measures, standardized heterozygosity was the only one that displayed significant global spatial autocorrelation, although this was in one year only (Figure

4.2). Standardized heterozygosity also produced more consistent patterns of local spatial autocorrelation (Figure 4.3; Table 4.3). Additionally, high and low ZGi* categories were more likely to represent significant differences in standardized heterozygosity values than mean relatedness to the colony (Table 4.4). Consistency of local spatial autocorrelation results in all years would suggest that distributions of high and low values were not random. However, patterns of local spatial autocorrelation for standardized heterozygosity appeared similar in only three out of four years (Figure 4.3). Positive correlations of local autocorrelation estimates between years were not significant after Bonferroni corrections though, suggesting patterns were not necessarily comparable (Table 4.3). This may be partially due to the differences in sampling between years, leading to only about a third of the sampled grid cells each year being compared. Patterns of local spatial autocorrelation of mean relatedness to the colony were only similar between two years (Figure 4.4) and most correlations were negative (Table 4.3). Thus, results for this measure were more inconsistent.

Variation in environmental conditions between breeding seasons could give rise to different patterns of habitat quality and thus genetic diversity and average relatedness. In particular, the size and abundance of pools varies between and within years depending on the amount of rainfall, which can alter the distribution of females (Redman et al. 2001; Twiss et al. 2002). In wet conditions, competition for sites is likely to be reduced compared to when conditions are dry and pools are scarce. In 2002, conditions were especially dry on North Rona early in the breeding season and different spatial patterns might have been expected. However, there was no significant relationship between the ease of access to pools and the local spatial autocorrelation of genetic diversity in this year (Table 4.7A). In general, variation in local spatial autocorrelation was best explained by the ease of access to the sea. This is not surprising as clusters of high and low values tended to group either on the eastern side of the Study Area (close to access points) or on the western side (far from access points) (Figure 4.3; 4.4). In agreement with the plots (Figure 4.3; 4.4), local spatial autocorrelation of genetic diversity produced more consistent results than mean relatedness to the colony, with estimates from all years, except 2001, having a negative relationship with cost distance to access points (Table 4.7A).

Mothers with higher local spatial autocorrelation values for genetic diversity (mothers at the centre of clusters of high genetic diversity) also pupped in areas with significantly higher density of females in 1997, and this trend was repeated in 2001 (Table 4.7A). There was also a significant positive relationship between the density of females and mothers' genetic diversity in two years (2000 and 2002, although only one year remained significant after Bonferroni corrections) (Table 4.7B). Thus, in all years, either genetic diversity, or local spatial autocorrelation of genetic diversity was positively correlated with female density. Conversely, in one year (2001) mothers with higher local spatial autocorrelation of mean relatedness to the colony were negatively correlated with density with this trend repeated in 1997 and 2000. However, in no year was there any significant relationship between density and mean relatedness to the colony.

Mothers may prefer to pup in areas of higher female density to reduce harassment by males (Boness et al. 1995). Additionally, density is usually a good predictor of habitat quality, as many individuals have chosen to breed in that location (Holmes et al. 1996; Petit & Petit 1996; Silverin 1998). On North Rona, female density was higher in locations where ease of access to water, through pools and access points to the sea, was greater (Table 4.6). It is possible that density is a better indicator of habitat quality on North Rona than topographic measures, as the cost-distance to pool measure may not include small-scale variations. Pools were measured in 1994 and, although the distribution of major pools is still similar, there have been changes in the location and size of smaller pools of water. Variation in weather conditions may also cause demand for water and its accessibility to change on an almost daily basis (Twiss et al. 2002). This fine-scale variation may be detected by patterns of density measured on the day a female gave birth. It should be noted that while females do crowd around pools of water, especially during relatively warm, dry conditions (Twiss et al. 2002), crowded conditions also occur in access gullies where there may be high levels of disturbance and lower pup survival (Boyd et al. 1962; Twiss et al. 2003). However, as a result of these high densities, few samples were obtained from these regions, to avoid undue disturbance from sampling. Thus, most sampled mothers were in the part of the Study Area where crowding usually occurs around pools.

There was also a non-significant relationship between cost-distance to access and genetic diversity in 1997. The relationship between ease of access to the sea and habitat quality is not thought to be linear because, as mentioned above, major transit routes occur in the immediate vicinity of access points causing these to be areas of high disturbance where pup survival may be lower (Boyd et al. 1962; Twiss et al. 2003). Females that pup an intermediate distance from the sea, out of areas of high disturbance but within central localities, may be the most successful (Pomeroy et al. 2001). However, the quadratic model that best explained the genetic diversity of mothers in 1997 did not conform to this expectation, as mothers with low genetic diversity were found an intermediate distance from the sea (Figure 4.5). As this result was not statistically significant (at $p < 0.05$) and was not replicated in any other year, it is likely that this relationship is not biologically relevant.

Overall, there were no strong replicable relationships between genetic diversity and habitat quality. The prediction that females with high genetic diversity pup in areas of good habitat was based on the assumption that females compete for access to these areas and that females with high genetic diversity have greater competitive ability. There is some evidence that mothers in good quality habitat have greater reproductive success (Twiss et al. 2000a; Pomeroy et al. 2001). However, the cause and effects of habitat use are unknown (Pomeroy et al. 2001). In other words, are females in good habitat because they are better, more competitive females or are females more successful because they are in these sites, or both? Many factors affect breeding success, and the importance of habitat to female fitness in relation to these other factors, such as maternal size and experience, is as yet unknown. If competition for sites were weak, detecting patterns of genetic diversity resulting from competition would be difficult. This may also vary between years depending on the amount of available water, which would complicate results further.

Conversely, patterns of habitat use may reflect previous knowledge and experience of mothers. Thus, high quality habitat may be used by older dominant individuals, as has been found for several avian species (Andrén 1990; Holmes et al. 1996; Rendón et al. 2001). In support of this hypothesis, Twiss et al. (2000a) found that female grey seals in preferred habitat on the Isle of May were larger (in terms of length), which suggests they may also have been older, more experienced mothers (Pomeroy et al. 1999).

Furthermore, a relationship between genetic diversity and habitat quality would not be detected if genetic diversity were not a good predictor of female quality and competitive ability in grey seals. While reproductive success has been found to increase with genetic diversity, female success was estimated in terms of whether or not pups were sampled in a given year (Amos et al. 2001a). This may not necessarily translate into mothers with high genetic diversity being good competitors or better mothers.

The strength of association between genetic diversity and fitness traits is generally weak in the species studied thus far (Britten 1996; Slate & Pemberton 2002; Coltman & Slate 2003). Moreover, many studies have failed to detect an association between genetic diversity and fitness (David 1998; Rowe & Beebee 2001; Wang et al. 2002) and repeatability of studies is often low, especially when environmental conditions vary or different molecular markers are used (Thelen & Allendorf 2001; Slate & Pemberton 2002; Wang et al. 2002). To detect consistent fitness correlations using genetic diversity estimates from microsatellites, hundreds of individuals genotyped at tens of loci may be required (David 1998; Slate & Pemberton 2002; Coltman & Slate 2003). However, two studies of grey seals have found positive relationships between fitness traits and genetic diversity using only nine of the microsatellite markers used here and a similar number of individuals (Amos et al. 2001a; Bean et al. 2004). Several of these markers may be linked to loci that are under selection suggesting fitness correlations with genetic diversity in grey seals may not solely be due to inbreeding depression (Bean et al. 2004). Indeed, it is likely that, with only 11 microsatellite markers, heterozygosity only correlates weakly with the inbreeding coefficient (Balloux et al. 2004; Slate et al. 2004). Unfortunately, pedigrees are not available for this population and therefore this cannot be explored further.

This study used the simplest measures of genetic diversity, heterozygosity, which was standardized to account for incomplete genotypes. However, two other measures of genetic diversity have been developed specifically for use with microsatellite loci. The first, known as mean d^2, uses differences in the number of allelic repeat units to estimate genetic diversity (Coulson et al. 1998) and assumes a stepwise model of mutation (Kimura & Ohta 1978). It has been proposed that this measure is more sensitive to population admixture and better at detecting heterosis than individual

heterozygosity (Pemberton et al. 1999). However, recent analyses suggest it is poorer at detecting associations with fitness traits than individual heterozygosity (Coltman & Slate 2003) especially when large numbers of loci are used (Slate & Pemberton 2002). This has also been found in studies of grey seals (Amos et al. 2001a; Bean et al. 2004). Additionally, results from simulations revealed that individual heterozygosity outperforms mean d^2 in virtually all situations (Tsitrone et al. 2001). Mean d^2 also correlates less well with the inbreeding coefficient than individual heterozygosity (Hedrick et al. 2001; Markert et al. 2004). For these reason it has been recommended that mean d^2 should only be used instead of individual heterozygosity in very restricted circumstances (i.e. recent mixing of very large subpopulations) (Tsitrone et al. 2001; Goudet & Keller 2002), which are unlikely to be applicable to the North Rona breeding colony.

Another genetic diversity measure, designed specifically for use with microsatellites, is internal relatedness (Amos et al. 2001a). This weights each genotype by the frequency of the alleles involved and is similar to the Queller & Goodnight (1989) relatedness estimator, except two alleles of a single individual are compared instead of two individuals. However, studies, including those using grey seals, have found that it correlates strongly with individual standardized heterozygosity, producing essentially identical results (Amos et al. 2001a; Masters et al. 2003; Hoffman et al. 2004). Thus there appears little benefit to using this less tested approach over heterozygosity.

This study could not repeat the results by Pomeroy et al. (2001) using average relatedness of mothers to the colony. Specifically, there was no relationship between local spatial autocorrelation and cost-distance to access points in 1997 (Table 4.7A), as had been found by Pomeroy et al. (2001) for samples taken in 1996. These years had a comparable distribution of samples, as all three pupping regions were sampled, and thus should have achieved the most similar results. There were both significant positive (2002) and negative (2000 and 2001) correlations between local spatial autocorrelation of mean relatedness to the colony and cost-distance to access, when only the Study Area was included (Table 4.7A). Therefore, the relationship of local spatial autocorrelation to ease of access to the sea does not appear consistent between years. Furthermore, in no year was there a significant correlation between any habitat characteristic and average relatedness of mothers to the colony (Table 4.7B). This was not tested by Pomeroy et al.

(2001) but is important because females with higher (or lower) ZGi* values often did not have significantly higher (or lower) mean relatedness to the colony (Table 4.4). Pomeroy et al. (2001) also found that mothers in the high ZGi* group had pups with higher growth rates. This suggests that females with high mean relatedness to the colony, and therefore probably low genetic diversity (Table 4.1), were better mothers. However, mothers in the high ZGi* group may not actually have had significantly higher mean relatedness to the colony even though they were at the centre of clusters suggested by the local spatial autocorrelation analysis (Table 4.4).

In examining patterns of habitat quality, only access to water and density were considered. Other studies using habitat characteristics of grey seal pupping sites have also measured the effect of slope and elevation (e.g. Pomeroy et al. 2000b; 2001; Twiss 2000a; 2001; 2003). Elevation is closely correlated with cost-distance to access to the sea on North Rona (Pomeroy et al. 2001). As there are many studies implicating the importance of water to breeding females (Pomeroy et al. 2000b; Twiss et al. 2000a; 2001; 2002; Redman et al. 2001) ease of access to the sea appears to have more biological meaning. Females also require relatively flat terrain to breed (Pomeroy et al. 2000a; Twiss et al. 2001). However, there is currently an excess of suitable flat terrain on North Rona, and thus it is unlikely to be a limiting factor for mothers (Pomeroy et al. 2001).

There was no relationship in any year between genetic diversity and pupping date (Table 4.5). Although there were significant correlations between local spatial autocorrelation of genetic diversity and pupping date, these were not consistent (Table 4.5). This is surprising as females that pup earlier on North Rona are larger and have heavier pups with higher growth rates (Pomeroy et al. 1999) that are more likely to survive (Hall et al. 2001). However, while pupping dates of individual females are generally consistent between years (Pomeroy et al. 1999; Redman 2002; Chapter 3) pupping date may also be affected by age and depend on a female's history (Pomeroy et al. 1999). It is also possible that genetic diversity is not a good predictor of female quality, as discussed above. There was also no correlation between pupping date and mean relatedness to the colony (Table 4.5). However, there was a significant correlation between local spatial autocorrelation of mean relatedness to the colony in 1997 and

2000. This was not found by Pomeroy et al. (2001) in their analysis of mothers from 1996 suggesting this result is not constant between years.

In conclusion, this study found some evidence that spatial patterns of the genetic diversity of mothers are consistent between years and correlated to female density. However, the lack of relationship between genetic diversity and topographic measures of habitat quality and pupping date, and inconsistency of results between years means that no firm conclusion can be drawn. No relationship between habitat characteristics or pupping date and mean relatedness to the colony of mothers was found and nor were spatial patterns consistent. Although this study produced few conclusive results, correlations between these variables may be stronger in other species. For instance, patterns may occur in small, threatened populations where levels of inbreeding are high or in populations suffering from loss of habitat where there is high degree of competition for breeding sites. In these situations, understanding the distribution of genetic diversity and patterns of colony relatedness though space and time is crucial to conserve genetic variation within a population. Using spatial autocorrelation techniques to uncover patterns of genetic variation may prove a useful tool for conservation geneticists.

Chapter 5: The effect of relatedness and genetic diversity on aggression between breeding female grey seals, *Halichoerus grypus*

5.1 Introduction

When individuals are aggregated and resources are limited competition can be intense, resulting in high levels of aggression between conspecifics. Appropriate levels of aggressive behaviour can increase fitness by maximising fecundity and reproductive success (Wilson 1998; Dingemanse et al. 2004). However, aggression may be costly and can be minimised through the development of social relationships (Pusey & Packer 1997). Kin selection theory provides a framework to try to understand the behavioural decision-making processes behind these social interactions.

Kin selection theory predicts that individuals will be less competitive, and thus aggressive, towards kin if they can gain indirect fitness benefits from this behaviour (Hamilton 1964). That is, if the cost (c) of reduced competition and aggression by the donor provides a benefit (b) through increased fitness to the recipient with relatedness (r) such that $rb - c > 0$. It has now been found in numerous species that kin are less competitive and more amicable towards each other (reviewed in Holmes 1988; Waldman 1988). Reduced aggression between relatives has also been observed in a wide range of vertebrate taxa including captive Californian sea lions *Zalophus californianus* (Hanggi & Schusterman 1990), white nosed coatis *Nasua narica* (Gompper et al. 1997), red grouse *Lagopus lagopus scoticus* (Watson et al. 1994), and juvenile rainbow trout *Oncorhynchus mykiss* (Brown & Brown 1993). Nevertheless, many studies have found that the level of aggression is not affected by the relatedness of participants (e.g. Caley & Boutin 1987; Spong & Creel 2004; West et al. 2001). This may be due to several factors including local competition between relatives and poor kin recognition abilities (Griffin & West 2002; Spong & Creel 2004).

Variation in the genetic diversity of population members may also affect social interactions. The genetic diversity of an animal (measured using molecular markers) may reflect their level of inbreeding, as mating between closely related individuals increases genome-wide homozygosity of offspring (Hedrick et al. 2001; Hansson & Westerberg 2002). However, genetic diversity may also measure selection on loci that are linked to molecular markers (Hansson et al. 2001; Markert et al. 2004; Slate et al.

2004). Significant positive correlations between genetic diversity and life history traits, such as juvenile survival and lifetime reproductive success, have been found in numerous species (reviewed in David 1998; Hansson & Westerberg 2002; Coltman & Slate 2003). Fewer studies have examined the effects of genetic diversity on behaviours that are likely to affect fitness (but see Marshall et al. 2003). One of these behaviours is the ability to compete successfully with conspecifics. A few laboratory-based studies have found that extreme inbreeding reduces competitive ability and aggression in *Drosophila melanogaster* (Latter & Sved 1994) and *Mus domesticus* (Eklund 1996; Meagher et al. 2000). Lower genetic diversity may also reduce competitive ability of males in wild populations (Högland et al. 2002; Hoffman et al. 2004). Additionally, in a recent laboratory-based study, Tiira et al. (2003) found groups of highly heterozygous juvenile salmon (*Salmo salar*) were more aggressive than less heterozygous juveniles.

Aggression by mothers is often directed towards conspecifics if they pose a threat to offspring (Maestripieri 1992). In phocid breeding colonies, mothers must protect their offspring from other females, which can trample or bite pups (Le Boeuf & Campagna 1994). Specifically, mothers will attack approaching alien pups (Coulson & Hickling 1964; Reiter et al. 1981; McCann 1982), which may be a mechanism to reduce milk stealing (Le Boeuf & Campagna 1994). Mothers are more aggressive towards other females in the immediate postpartum phase when pups are most vulnerable (Christenson & Le Boeuf 1978; McCann 1982). Aggression then tends to decrease through the lactation period (Boness et al. 1982; Redman 2002). Aggression by a mother towards another female can minimise the chances of an attack on the pup by increasing the distance between the other female and the pup (Boness et al. 1982; McCann 1982). Furthermore, elephant seal (*Mirounga spp.*) mothers that displayed more aggression, and were more proficient at moving other females away from the immediate vicinity, were found to be more successful at raising a pup (Christenson & Le Boeuf 1978; Ribic 1988; Engelhard et al. 2002). In other species, including grey seals, mothers may defend a perimeter around their pup against other adults (Fogden 1971; Stewart 1987).

Phocid mothers may also use aggression towards other females to gain and protect good quality pupping sites within a breeding colony. Females with lower competitive ability that are subordinate or inexperienced may be forced to pup in poorer quality habitat

where pup survival is lower (Reiter et al. 1981; Ribic 1988; Hastings & Testa 1998; Twiss et al. 2000a). Furthermore, Christenson & Le Boeuf (1978) reported that in crowded elephant seal breeding colonies, aggression by mothers toward approaching females resulted in lower density sites where pup survival was higher. Therefore, pup protection and competition for pupping sites are probable functions of female-female aggression in phocids.

However, female aggression can be costly to phocid mothers in terms of their pups' survival and growth. During an aggressive interaction, pups can move away and become separated from their mothers (Boness 1990; Le Boeuf & Campagna 1994). Permanent mother-pup separation is more likely when the pup is very young and the mother-pup bond has not yet formed (Burton et al. 1975). Separation from the mother is a major cause of death for pups, as they do not get protection, and will almost certainly starve (Anderson et al. 1979).

Aggression by mothers may also have an energetic cost. Phocid mothers are dependent on stored energy reserves and, in many species, do not feed during their brief lactation period (Lydersen & Kovacs 1999). In species that rely solely on stored energy, mothers spend most of their time resting, which may be an adaptation to conserve energy (Anderson & Harwood 1985; Kovacs 1987). Total daily energy expenditures are high compared to many lactating mammals due to high levels of milk production (Mellish et al. 2000). Mothers that experience high levels of aggression may have higher stress levels or have less energy to pass on to pups, both of which could reduce milk quality. Aggression can also distract from the process of lactation by disrupting suckling bouts or causing fewer suckling bouts to be initiated, which could result in less energy being passed to pups (Fogden 1971).

Finally, aggression could be costly to mothers through injury. However, aggression between phocid females usually takes the form of threats; prolonged contact, where injuries are likely to occur, is rare (McCann 1982; Kovacs 1987; Stewart 1987). Thus aggression towards another female usually represents a minimal risk to the mother in terms of injury.

In grey seals, rates of aggression between breeding females are typically low, although this varies with the topography of the colony (Anderson & Harwood 1985; Kovacs 1987; Haller et al. 1996; Caudron et al. 2001). For example, Redman (2002) found that females on the Isle of May had higher rates of aggression than those on North Rona. This is probably due to differences in the distribution of available water causing greater movement of females on the Isle of May, which increases disturbance. Topographies that result in restricted numbers of females crowding together, such as caves, can also increase aggression (Caudron et al. 2001). Conversely, grey seals breeding on land-fast and pack ice tend to have relatively low rates of aggression (Haller et al. 1996).

In the North Rona breeding colony, females spend only 0.6% of their time involved in aggressive interactions with other females (Anderson & Harwood 1985). In typical years, mothers in this colony rarely return to the sea during lactation and instead cluster around inland pools of water (Boyd et al. 1962; Anderson et al. 1975; Pomeroy et al. 1994), which they may use to reduce thermal stress (Twiss et al. 2002). Availability of water is thought to be the most important feature in pupping site selection (Pomeroy et al. 2000b; Twiss et al. 2000a; 2001; Redman et al. 2001). Thus, competition may arise for access to pools of water, resulting in higher levels of aggression in these areas.

As relatives are often less competitive with each other (Holmes 1988; Waldman 1988), females on the North Rona colony were expected to reduce aggression when competing with relatives. Higher levels of aggression towards non-relatives, when competing for prime pupping sites, could result in relatives pupping together in high quality sites. Although there is little evidence of kin clustering on North Rona (Pomeroy et al. 2001; Chapter 3) the extent of kin clustering may vary depending on site quality. Aggression rates may also vary with genetic diversity, if females with high genetic diversity have greater competitive ability or these mothers are better at protecting their pups from other females.

5.2 Aims

The aim of this chapter was to determine whether relatedness or genetic diversity moderated behaviour between female grey seals in the North Rona breeding colony. The behaviour used to investigate this was aggression, as it is the most commonly observed and easily identified interaction between females. Other factors that may also affect aggression, including the year, site and age of the pup, were also investigated. To study the direct behavioural causes and outcomes of aggression, behaviours that occurred before and after each interaction were examined.

Females were predicted to be less aggressive towards their relatives. This was tested by i) comparing the relatedness of pairs of females that were observed having aggressive interactions and those that were not and ii) for each female, comparing the relatedness of those neighbours she was and was not observed interacting aggressively with. The relatedness and behaviour of females that remained in the central focal areas and those that moved through the area were also compared. Female residents within focal areas were predicted to be more related to each other and be more aggressive to unrelated females that moved through the area. Females that had higher genetic diversity and lower relatedness to their neighbours were predicted to initiate aggressive interactions more often. Other factors that were hypothesised to increase the rate of a female's aggressive interactions were density, closeness to pools of water and movement within focal areas.

5.3 Methods

5.3.1 Behavioural observations

Hides were set up overlooking the Study Area (Figure 3.1) such that seals could be readily seen with binoculars. Focal observations were made on groups of seals within two geographically defined areas (Figure 5.1). A female became a focal female when she entered a focal area. In 2001 observations were made on the West Pools (WP) only (Figure 5.1; Table 5.1). In 2002 observations were made on the East Pools (EP) (Figure 5.1; Table 5.1) and the WP, with a larger area being watched than in 2001 (Table 5.1). The WP was observed for between six and eight hours per day in 2001 while in 2002 both the WP and the EP were observed for four hours per day (one in the morning and one in the afternoon, alternating morning and afternoon sessions). However, for four days at the end of the 2002 season, observations were made exclusively on the WP for

eight hours a day. Females were observed in October and November (Table 5.1). Observations started on a focal area either at the start of the field season or when females were first present in the area. Observation ceased when fewer than three females were present or the field season ended. Observations were made on approximately 60% of all possible days (Table 5.1) with other days being used to collect genetic samples from animals in the colony.

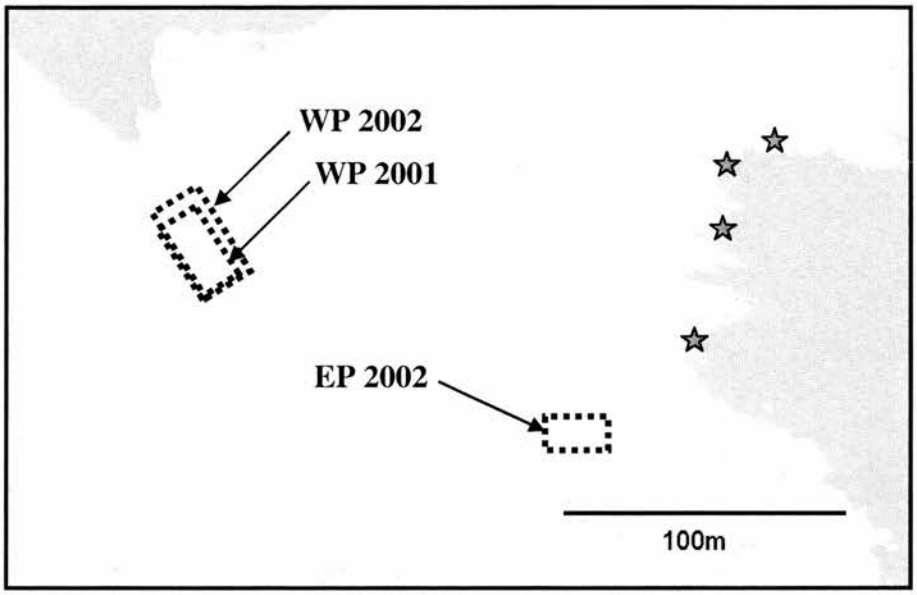


Figure 5.1: Locations of the West Pools (WP) and East Pools (EP) focal areas with boundaries for each year (dotted lines) drawn roughly to scale. These are drawn on a map of the Study Area, which forms part of the Fianuis peninsula on North Rona. Land is shown in white and sea is shown in grey. Access to the colony is limited to several gullies (indicated by stars) on the eastern side of the peninsula.

Table 5.1: Approximate size of the focal areas and the duration of observations.

Area	Year	Size (m^2)	Dates observed	Days observed	Hours observed
WP	2001	450	09/10 – 06/11	16	123
WP	2002	600	01/10 – 05/11	24	112
EP	2002	200	01/10 – 28/10	18	72

Focal areas were chosen because they contained pools of water and were used each year. The EP was closer to access gullies leading to the sea than the WP, which was reached by females traversing most of the Study Area (Figure 5.1). Before observations began, boundaries of the focal areas were delineated onto on a detailed map of the Study Area with an overlaid 10 m interval Ordinance Survey grid (as in Twiss et al. 2001). Boundaries were chosen so they could be identified from topographic features on

the ground and to minimise areas that were not visible from the hides (i.e. behind ridges).

Locations of all focal females were recorded every hour to within 2 m accuracy using a standard geo-rectified map. The locations of pools of water within the focal areas were also recorded on the map at the beginning of the season and pools were added when they appeared in the course of the observational period. The stage of each female's pup (Table 3.1) was also recorded daily. Focal females could be recognised from day to day using pelage markings (Redman 2002) although a few also had brands or flipper tags. Sketches were made of pelage markings of all focal females and photographs taken when possible (by S. Ruddell). However, for females that were only in the focal area for a brief period of time, or when pelage was obscured (due to positioning or unclean pelage), sketches were sometimes of sufficiently poor quality that females could not be reliably identified again. Thus it is possible that occasionally the same female was given two unique identities. Females that were not identified could not be sampled for genetic analysis and thus were not included in many analyses (see below). Between years, focal females were compared to those present in the North Rona female pelage database by comparing photographs with pelage markings of females in the field. All instances of putative matches between females were confirmed by two observers. Some matches were made retrospectively through pelage or genetic matches (see Chapter 2 for details). Newly identified females were added to the pelage database.

Continuous observations of all behaviours of focal females were recorded on data collection sheets. When an aggressive interaction occurred between females the following was recorded: time, date, identity of initiator and recipient, and behaviour of each participant. All interactions reported were aggressive interactions. Interactions were divided into four categories based on the intensity of aggression; this was measured according to the presence of a behaviour. Categories are listed below in increasing order of intensity:

1) Low level threat - Open mouth threat only (a female opens her mouth with whiskers erect towards the recipient and often vocalises).
2) High level threat – One of the following was observed during the interaction: lunging (a female swiftly extends her neck and head towards the recipient) or flippering (a female rapidly moves a fore-flipper).

3) Low level contact - Contact is made briefly between females.
4) High level contact - Contact occurred repeatedly during the interaction.

Following Redman (2002), if an interaction stopped and then started again, one bout was recorded provided there was less than one minute between the interactions and there was no obvious outcome.

A behaviour was classified as preceding an interaction only if it was recorded within the minute before the interaction took place. Preceding behaviours were recorded separately for the initiator and the recipient. When a behaviour was observed it was categorised into four types:

1) Approach – A female moved directly towards the other female.
2) Transit – A female moved but not directly towards the other female.
3) Interact – A female interacted aggressively with another female or a male.
4) Maternal – A female performed a maternal behaviour including sniffing, presenting the nipples to, or suckling the pup.

A behaviour was classified as following an interaction only if it was recorded within the minute after the end of the interaction. Behaviours were recorded separately for initiators and recipients. When a behaviour was observed it was categorised into four types:

1) Turn away – A female turned her head away from the other female or shifted orientation but remained in the same location.
2) Move away – A female moved at least one body length away from the other female.
3) Interaction – A female interacted aggressively with another female or male
4) Maternal – Either a female turned or moved away but the behaviour was directly towards her pup or a female performed a maternal behaviour.

Neighbours were defined as the subset of individuals with which a female was likely to interact. To identify this subset, for each observed interaction, the distance between the initiator and recipient was calculated from their xy coordinates recorded at the hourly observation event closest to the time of the interaction. This was performed only for the 2002 data, so each focal area was only represented once. Females were within 10 m of

each other in 95% (452/476) of interactions (Figure 5.2). Thus all females within a 10 m radius were considered as neighbours, as this only excluded 5% of interactants. However, for 16% (91/567) of interactions, one of the interactants was not recorded as being present in the focal area at the closest hourly interval. This is unavoidable, as females moved in and out of the focal area (i.e. a female may enter the area at five minutes past the hour and have an interaction). Thus, on average, neighbours at any particular hour (that is all females within 10 m) will represent 79% of interactants. This measure of a neighbour was used instead of a female's nearest neighbour, which could be considered the only individual a female was likely to interact with. However, the recipient of a female's aggression was her sole nearest neighbour in only 38% of interactions, as measured hourly. This indicates relatedness to the nearest neighbour was not a representative measure of the relatedness of a female's interactants and therefore was not used.

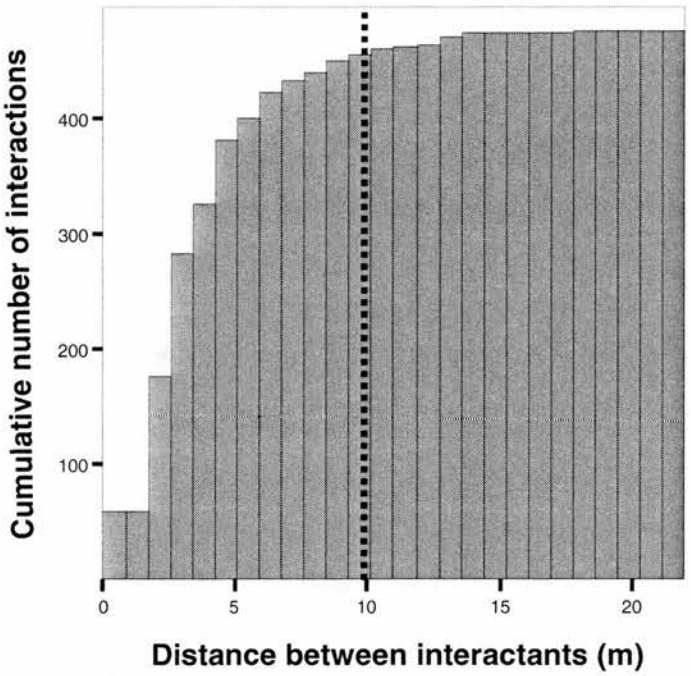


Figure 5.2: Cumulative histogram of the distance between interacting females at their nearest hourly distance measurement. Ninety-five percent of the time interactions took place within 10 m, indicated by the dashed line.

5.3.2 Genetic sampling and relatedness estimates

Samples were collected and genotyped as described in Chapter 2. Every effort was made to obtain genetic samples from all focal females. However, sampling inevitably disturbs mothers and pup and to avoid this as much as possible sampling within focal

areas was limited to a few times per season. Some females were not sampled because either i) sketches were of poor quality, so females could not be recognised again or ii) recognisable females left the focal area but were not resighted or were in areas where sampling would have cause unacceptable levels of disturbance to the colony. Relatedness between females was estimated using RELATEDNESS 5.0 (Queller & Goodnight 1989; Chapter 2). Genotypes from all individual adults sampled between 1997 and 2003 (n = 437) were used to generate background allele frequencies. The allele frequencies used to estimate relatedness should not include related females (Queller & Goodnight, 1989), which is undoubtedly the case here. However, it is virtually impossible to avoid this for populations with unknown pedigrees and the large number of individual adults included in the sample should ensure a reasonable approximation of the actual allele frequencies of adults on North Rona.

5.3.3 Effect of non-genetic variables on aggression

A seasonal interaction rate (per hour per female) for each focal area was calculated as: total number of aggressive interactions observed / (median number of females observed per day x total number of hours observed), following Redman (2002).

The number of interactions observed per day was compared between the WP in 2001 and 2002 and between the WP and EP in 2002 using general linear models (GLMs). The number of hours and the total number of focal females observed per day were also included as explanatory variables. To test for an association between the observed frequency of each category of aggressive behaviour and the focal area or year, a χ^2 test was performed using a contingency table. To investigate which behaviours were more frequently performed by initiators versus recipients, both preceding and following aggressive interactions, separate χ^2 tests (with Yates correction) were performed for each behavioural category to test for deviation from a predicted 50/50 split. Results from all focal areas were combined. Although this analysis will suffer from some pseudoreplication, as multiple results for some females were included, a sufficiently large number of different females' behaviour was recorded that these tests should give a robust overall indication of the patterns occurring in the focal areas.

If most maternal aggression occurs to protect pups, then aggression is expected to decrease as pups get older and less vulnerable. To test for this association, the number

of interactions initiated per hour by focal females when their pup was stages I, II, and III or greater was compared (Table 3.1). Analysis was performed using a general linear mixed model (GLMM) using a restricted maximum likelihood method with 100 iterations. The rate of initiated interactions was transformed with $-1/(x+1)$ to achieve normality. Only females that were observed with their pup during all three stages were included. Female was entered as a random factor and pup stage as a repeated measure. The focal area or year (included as one variable with three categories) and median number of neighbours (measure hourly) at each pup stage were included as explanatory variables. Non-significant variables were removed from the model in a backwards stepwise manner. Residuals were tested for normality and plots of residuals versus fitted values were checked for homogeneity of variance.

5.3.4 Effect of relatedness on aggression

Analyses in the following section were performed separately for each focal area and year. The distribution of relatedness of focal females from each focal area was compared to the distribution of 1000 unrelated pairs simulated in Kinship 1.0 (Queller & Goodnight 1999) from North Rona allele frequencies (taken from Chapter 2) using StatXact (Cytel Software Corporation). A pair was only included in analyses if they were recorded as present in the focal area at the same time at least once. The relatedness of pairs that were observed interacting at least once was compared to those that were not, for pairs that were within a median of 10 m of each other. For pairs that were observed interacting, those with higher relatedness may have tended to have lower intensity interactions. Thus, for interactants, the highest intensity of aggression that either member of pair displayed was determined and relatedness of pairs interacting at each level compared.

There was variation in the amount of time females were present in the focal areas, with some females observed for only a day (transients). These females may not have remained in the focal areas because of their relatedness to, or interactions with, females already present. To investigate this possibility, the distribution of relatedness was compared between pairs where i) both females were present for > 1 day and ii) one female was present for > 1 day and one female was a transient. The relatedness of pairs that were observed interacting at least once and those that were not was also compared for the two categories listed above. All pairs were included, regardless of their median

distance apart, to maintain sample sizes. It should be noted that in these analyses transients were defined as females observed for only one day. However, as focal areas were not observed every day, these females may have been present for longer.

Spurious patterns of relatedness and aggression could arise if related females were not randomly distributed with respect to space and time (i.e. if relatives were closer to each other they would be expected to interact more often). To test for this, correlations between the relatedness of pairs and their i) median distance apart (measured hourly) and ii) hours observed together were tested using Spearman's rank coefficients (using StatXact). While correlations between pairwise data are usually performed using Mantel tests (as in Chapter 3), they were not used here since most pairs of females were not in the focal area together resulting in the majority of the matrix containing missing data.

For all analyses using pairwise data (i.e. median distance between a pair, number of hours a pair were observed together in the focal area, mean relatedness of a pair, and number of times a pair interacted) non-parametric statistics were used. As pairwise estimates are not independent, significance of pairwise analyses was tested using 10 000 Monte Carlo simulations by repeat sampling from the relevant data set. Simulations were performed in SPSS 11.0 unless otherwise specified.

As a complementary analysis to the pairwise approach, the relatedness of each female to those neighbours she interacted with was compared to those neighbours she did not interact with. The mean relatedness of the neighbours that a female initiated an aggressive interaction with was calculated daily. Similarly, the mean relatedness of neighbours that an interaction was not initiated with was calculated daily. The seasonal mean of these two categories was taken for each female. The difference in mean relatedness between those neighbours a female initiated interactions with, and those she did not, was compared using a sign test.

5.3.5 Analyses of individual interaction rates

A GLM was used to test whether relatedness of neighbours or heterozygosity of a female affected her rate of aggressive interactions. Only females that were genotyped were included in the model. As in the GLMM for pup stage, the response variable for each female was the number of initiated aggressive interactions divided by the number

of hours observed, transformed with $-1/(x+1)$ to achieve normality. The model was weighted by the number of observation hours for each female to differentiate between the amounts of information each female provided. This was not done in the pup stage model because there was considerably less variation in the number of hours observed, as only a subset of females was used in that analysis. The two years (2001 and 2002) were analysed separately because many of the same individuals were present in both years (see results). There were two females that were present in both the WP and EP focal areas in 2002. In each case, the female was removed from the focal area with the lower number of observations, to avoid replication.

Six continuous and two categorical explanatory variables were included in the model. The six non-genetic variables were included because they were considered to be potentially important in explaining aggression and thus needed to be taken into account when examining the effects of relatedness and heterozygosity. The eight variables were as follows:

1) Relatedness: the mean relatedness of all neighbours calculated daily, averaged over the season. A female was considered as a neighbour if she was recorded within 10 m of the focal female at least once during hourly measurements. Since relatedness is pairwise, a relatedness estimate between neighbours will be included in both of the females' daily mean relatedness estimates. This could create problems with independence. However, most females had many neighbours, which will increase independence between the estimates. Thus, females that had two or less neighbours were removed from analysis (2001 1/30; 2002 3/70).
2) Heterozygosity: this was used as an estimate of genetic diversity and was calculated as the proportion of loci for which a female was heterozygous. Heterozygosity was not standardized (as in Chapter 4), as all focal females were genotyped at all 11 loci.
3) Density: the median number of neighbours, measured hourly.
4) Distance to pool: the median distance to the nearest pool of water, measured hourly. A pool was included if it was greater than seal size or approximately 0.68 m^2 (female bodies are approximately 1.39 m long and 0.49 m wide at their widest point, as measured from aerial photographs, Twiss et al. 2000b). Only these larger pools were included as they were considered as potential foci of

high aggression, since they were used by more seals. The sizes of pools were estimated from aerial photographs taken (by C. Duck) in the SMRU aerial survey on the following dates in 2001: 04/10, 16/10, 01/11 and 2002: 05/10, 18/10, 27/10, 02/11. As the presence of pools changed over the season (pools were larger and more abundant later in the season) for each day the aerial photograph taken closest to that date was used.

5) Movement: the sum of movement between hours within the focal area, divided by the number of observation hours.
6) First Day: the first day recorded as a focal female. Pupping date (the day a female was seen giving birth or the date estimated from the age of the pup, see Chapter 3) was also fitted in the model for females that were observed with a pup during the breeding season. However, this explained less variation in the model than the first day and so was not included. Pupping date and the first day in the focal area were strongly correlated (Spearman's correlation 2001 $r = 0.846$, $n = 26$, $p < 0.001$; 2002 $r = 0.951$ $n = 63$, $p < 0.001$) for females with pups (2001 26/29; 2002 63/67). It is probable that first day as a focal female explained more variation because females occasionally pupped several days after their first observation day and sometimes outside the focal area.
7) Resident / non-resident: Resident females were those that were observed in the focal area for at least two days and were present with a pup for some of that time. Non-resident females were those that were either observed for just one day in the focal area or were never observed with a pup.
8) Focal area: the focal area (WP vs. EP) in which a female was observed (for the 2002 model only).

Given that so many main effect terms were entered into the model, only interaction terms including genetic variables (i.e. relatedness and heterozygosity) were fitted. The model was reduced using a backwards stepwise procedure by sequentially removing non-significant interaction terms that explained the least variation to $p < 0.05$. Main effect terms were then removed sequentially only if this did not cause a decrease in the adjusted r^2 value. Residuals from the model were tested for normality using Kolmogorov-Smirnov tests. Levene's test of equality of error variances was used to test for homogeneity of variance. Additionally, plots of standardized residuals versus fitted values were examined to ensure there was an even scatter of residuals across the range

of fitted values. Significant interaction terms between continuous variables were visualised by plotting them against back-transformed predicted values taken from the model. The surface of the plot was fitted with a smoother function (local linear regression) using a normal kernel. Statistical analyses were performed using SPSS 11.0 unless otherwise stated.

5.4 Results

5.4.1 Comparison of focal areas and years

The total number of focal females observed over the season was similar in the two focal areas and years (Table 5.2). There was no significant difference in the number of focal females observed each day i) in the WP in 2001 and 2002 ($t = 1.48$, d.f. = 39, $p = 0.148$) or ii) in 2002 in the WP and EP ($t = 1.53$, d.f. = 40, $p = 0.134$; Table 5.2). Approximately half of all focal females were observed for only one day (WP 2001 55%; WP 2002 50%; EP 2002 51%). For the remaining females, the number of days present tended to have a bimodal distribution (Figure 5.3). This trend was most apparent in the EP in 2002 where no females were observed for six to eight days. Bimodality tended to represent a divide between those females that were observed for their complete lactation period and those that were not. Some females moved outside the focal areas during lactation while other focal females had late pupping dates (in the WP only) and were still lactating when observation ended. As expected from the different dates the focal areas were observed in the two years (Table 5.1), females in the WP in 2001 had significantly later pupping dates than those in 2002 ($t = 2.10$, d.f. = 67, $p = 0.039$). In 2002, females in the EP had significantly earlier pupping dates than those in the WP ($t = 4.90$, d.f. = 73, $p < 0.001$).

Table 5.2: Description of numbers of females and the proportion of genotyped females in each focal area and year. The proportion of females with genotypes is presented for all females and then separately for females that were observed for more than one day and for one day only. Numbers in brackets are the number of females genotyped / the total number of females in that category.

Area	Year	Number of females		Proportion Genotyped		
		Total	Daily mean ± SD	All	>1 day	1 day
WP	2001	53	10.05 ± 2.99	0.57 (30/53)	0.92 (23/25)	0.25 (7/28)
WP	2002	68	12.38 ± 5.95	0.60 (41/68)	0.91 (31/34)	0.29 (10/34)
EP	2002	49	10.00 ± 3.25	0.62 (30/49)	0.92 (22/24)	0.32 (8/25)

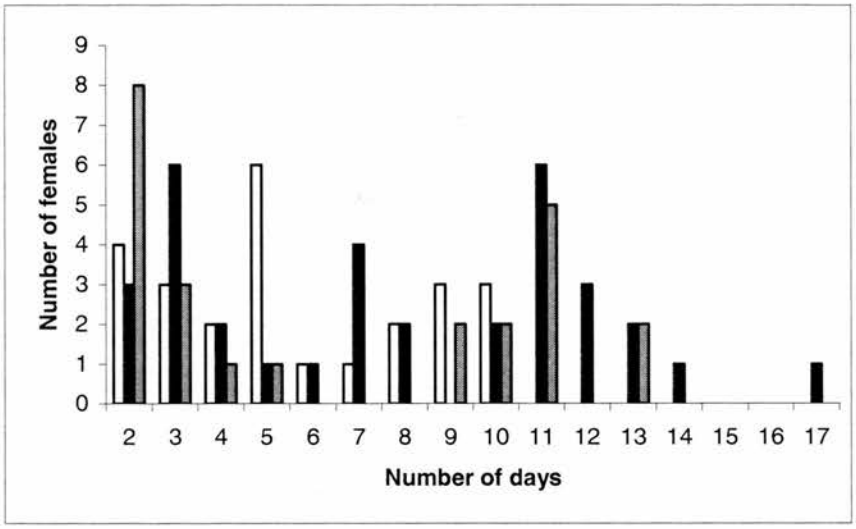


Figure 5.3: Number of days focal females were observed, for females observed for at least two days, in the WP 2001 (white bars), WP 2002 (black bars) and EP 2002 (grey bars).

Of the focal females observed in the WP in 2001, 11 were also observed in the WP in 2002. All of the 11 females were genotyped, resulting in 37% (11/30) of those in the WP 2001 dataset being in the WP 2002 dataset. However, only five were present for more than one day and had a pup in both years. In 2002, one of the five was present for only two days with a pup and one lost her pup after two days of observations. Thus, there were too few females in this dataset to make direct comparisons of breeding behaviour between years. Of the remaining 18 focal females from the WP in 2001 that were entered in the female pelage database, 11 were observed elsewhere on the colony in 2002; one was a focal female in the EP.

Over 90% of females that were observed in the focal area for more than one day were sampled and genotyped (Table 5.2). Inevitably, it was more difficult to obtain samples from females that were observed for one day only and thus a smaller proportion were genotyped (Table 5.2). Similar proportions of females were genotyped in all focal areas, although more females that were observed for a single day were genotyped in 2002 (Table 5.2). This incomplete sampling resulted in a proportion of females being missing from each calculation of mean relatedness of neighbouring females (used as an explanatory variable in the GLM of interaction rates). The mean proportion of females missing from each calculation of mean relatedness of neighbouring females was 0.14 ± 0.09 SD in 2001 and 0.12 ± 0.09 SD in 2002. There was no significant difference in the proportion missing between the two years (t-test: $t = 1.04$, d.f. = 94, $p = 0.302$).

5.4.2. Characterisation of aggression

A total of 699 aggressive interactions were recorded for the three focal groups (Table 5.3). Seasonal interaction rates (per hour, per female) were lower in 2001 than in 2002 (Table 5.3). The daily number of interactions was significantly lower in 2001 than in 2002 ($F_{1,36} = 9.60$, $p = 0.004$), but there was no difference between focal areas in 2002 ($F_{1,38} = 1.18$, $p = 0.284$; Figure 5.4). Between years, the number of interactions increased with the number of hours ($F_{1,36} = 6.69$, $p = 0.014$) and the number of focal females observed each day ($F_{1,36} = 16.84$, $p < 0.001$). Between focal areas, the number of interactions increased with the number of focal females ($F_{1,38} = 16.03$, $p < 0.001$) but not with the number of hours observed daily ($F_{1,38} = 2.50$, $p = 0.114$). This non-significant result probably occurred because there was little variation in the number of hours observed, as all days except four were observed for four hours.

Table 5.3: Recorded number and seasonal rate of aggressive interactions in each focal area and year.

Area	Year	Aggressive interactions	Interaction rate
WP	2001	132	0.10
WP	2002	334	0.25
EP	2002	233	0.32

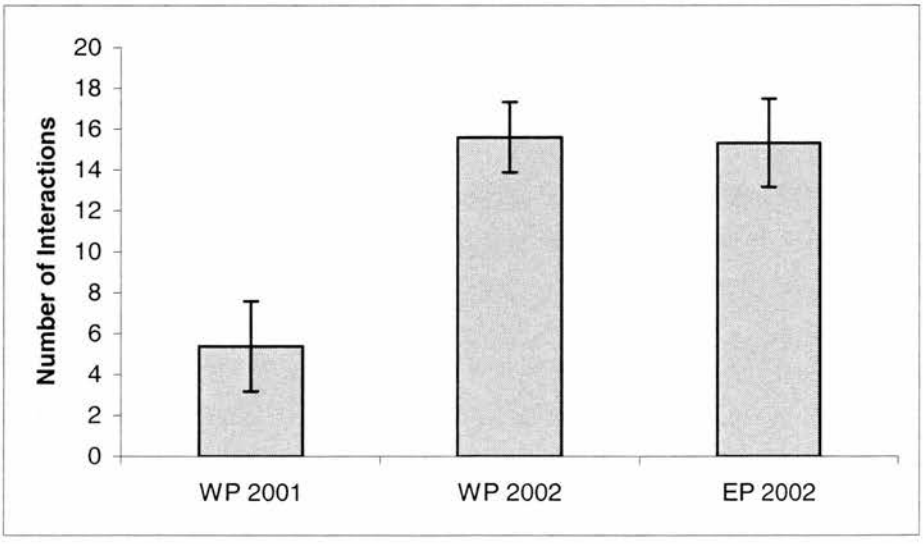


Figure 5.4: Fitted means ± SE of daily counts of interactions in the focal areas from general linear models.

No high level contact interactions were recorded in either year. Few interactions were recorded that involved low levels of contact (n = 13) and all of these occurred in 2002 (Table 5.4). A similar number of high and low level threats were recorded daily (mean ± SD; high 5.44 ± 5.41, low 6.19 ± 5.18). There was a significant association between the focal area or year and the frequency of each level of interaction (χ^2 = 15.62, d.f. = 4, p = 0.002; Table 5.4). This was mainly due to i) more high level threats and fewer low level threats and low level contacts than expected in 2001 and ii) more low level threats and fewer high level threats than expected in the WP in 2002 (Table 5.4).

Table 5.4: Counts of observed interactions at each level of intensity for each focal area and year. Numbers in brackets are expected values.

Area	Year	Low level threat	High level threat	Low level contact
WP	2001	53 (69)	79 (61)	0 (3)
WP	2002	190 (174)	137 (153)	7 (6)
EP	2002	122 (122)	105 (107)	6 (4)

No behaviour was recorded for either the initiator or the recipient before just over half of all interactions (53%). Approaching females were initiators of aggression significantly more often than they were recipients while recipients of aggression were more often in transit (Table 5.5). Initiators also performed maternal behaviours significantly more frequently than recipients before an interaction (Table 5.5). Females may behave differently in interactions if they have a pup. For instance, females with pups may be more likely to approach and initiate aggressive interactions with a female they perceive as a threat. Conversely, females that approach without a pup may more likely to be recipients of aggression. However, approaching initiators were no more likely to have pups (86%) than approaching recipients (76%; χ^2 = 2.02, d.f. = 1, p = 0.102). Similarly, there were an almost equal proportion of initiators (73%) and recipients (72%) with pups in transit.

Following an interaction, 58% of the time no behaviour was recorded for either the initiator or the recipient. Recipients moved away significantly more often after an interaction than initiators and tended to turn away more often (Table 5.5). Both were equally likely to perform a maternal behaviour or interact with another adult, although this was a rare occurrence. For those that moved away after an interaction, there was no difference between initiators and recipients in the proportion of females with pups (initiators 71%, recipients 82%; χ^2 = 1.03, d.f. = 1, p = 0.235).

Table 5.5: Comparisons between the behaviour of females that initiated aggressive interactions and those that were recipients, using χ^2 tests.

Behaviour	Count		χ^2	p
	Initiator	Recipient		
Preceding				
Approach	112	67	11.31	<0.001
Transit	22	57	15.51	<0.001
Interact	33	39	0.50	0.480
Maternal	28	11	7.41	0.006
Following				
Move away	34	93	27.41	<0.001
Turn away	38	56	3.04	0.081
Interact	4	6	0.40	0.527
Maternal	35	41	0.47	0.491

There were 36 females that were observed over the season with pups in at least three different stages. None were present in both 2001 and 2002. There was a non-significant trend for females to initiate fewer interactions as pup stage increased ($F_{2,52.28} = 2.54$, $p = 0.089$; Figure 5.5). The number of interactions increased with the median number of neighbours ($F_{1,37.21} = 14.67$, $p < 0.001$). The focal area/year variable was not retained in the model. Density co-varied with focal area/year (one way ANOVA: $F_{2,105} = 28.32$, $p < 0.001$) and, since both explained the same variation in the model, only one was retained.

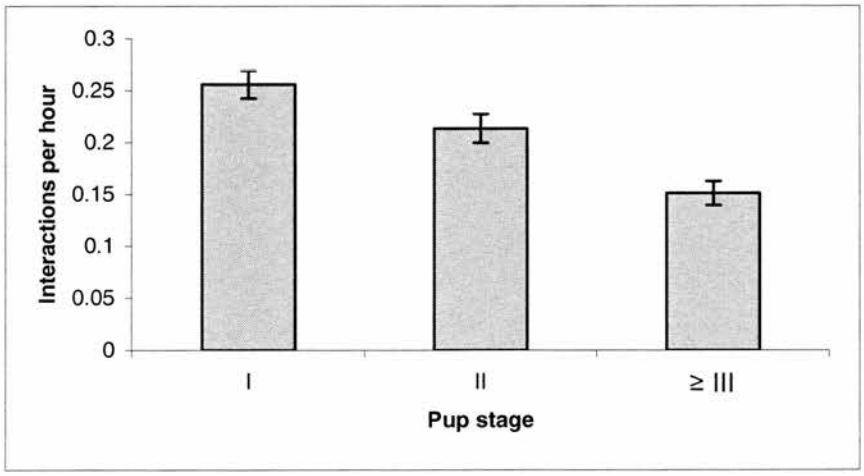


Figure 5.5: Back transformed predicted means ± SE of rates of aggressive interactions at each pup stage from a generalized linear mixed model of interaction rates.

5.4.3 Effect of relatedness on aggression

The distribution of relatedness of pairs of focal females (recorded together in the focal area at least once) was similar for all focal areas (Figure 5.6). In no year or focal area did the distribution differ from the distribution of simulated unrelated pairs (Figure 5.6) with mean and standard deviation 0.00 ± 0.158 (Kolmogorov-Smirnov one-sample tests: WP 2001 Z = 0.53, p = 0.562; WP 2002 Z = 0.53, p = 0.564; EP 2002 Z = 0.22, p = 0.890). Only 15% (130/840) of female pairs were related according to the relaxed definition of relatedness (r > 0.155) and only 2% (19/840) according to the strict definition (r > 0.314) (as described in Chapter 2). No female pairs had r-values of 0.5 or higher (the average value expected for full siblings and parent – offspring pairs) (Figure 5.6).

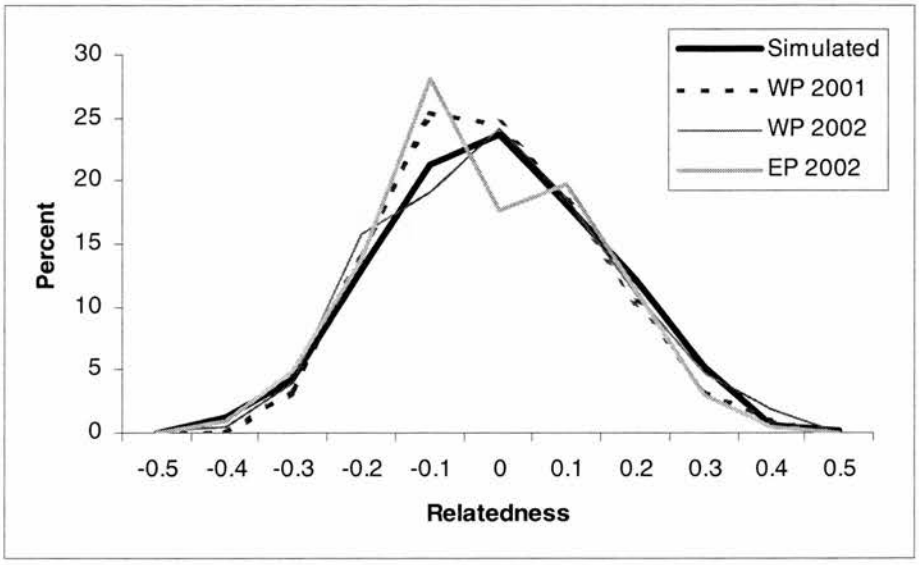
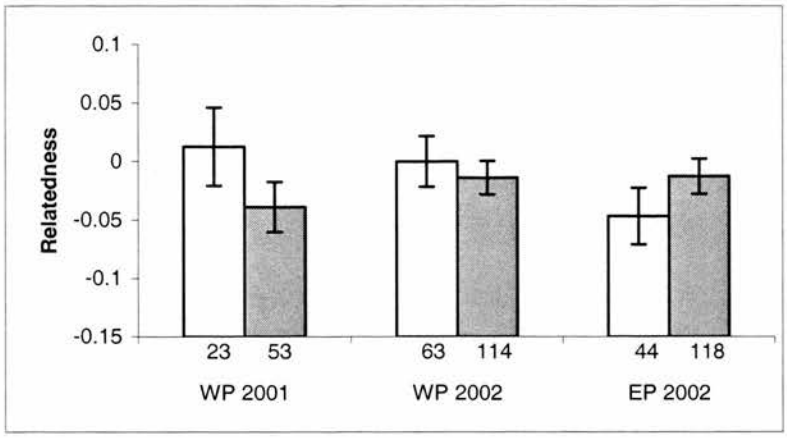


Figure 5.6: Distribution of relatedness estimates of focal females (WP 2001 n = 213, WP 2002 n = 425, EP 2002 n = 202) and for 1000 simulated unrelated pairs. Numbers of individual females genotyped in each year are listed in Table 5.2.

There was no difference between the relatedness of pairs that were observed interacting and those that were not (Figure 5.7A; Mann-Whitney U tests: WP 2001 Z = 1.41 p = 0.162; WP 2002 Z = 0.74, p = 0.459; EP 2002 Z = 1.26, p = 0.209). Furthermore, pairs that displayed a lower intensity of aggression were neither more nor less related than those that displayed a higher intensity (Figure 5.8; Mann-Whitney U test: WP 2001 Z = -1.28 p = 0.217; Kruskal-Wallis tests: WP 2002 $\chi^2 = 0.61$, p = 0.752; EP 2002 $\chi^2 = 0.61$, p = 0.748).

A



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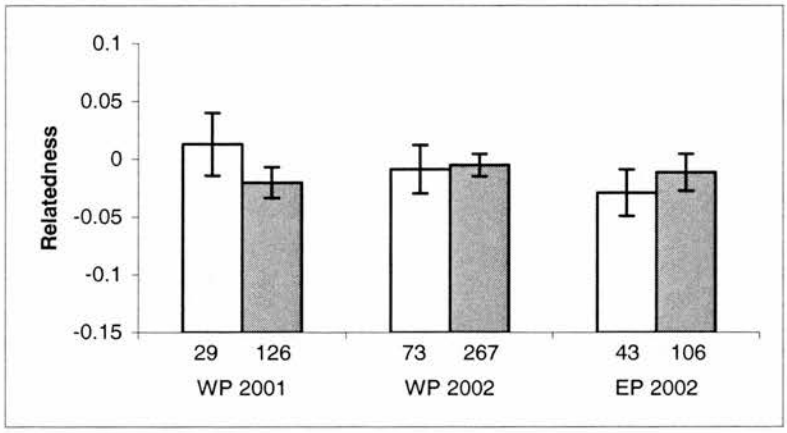

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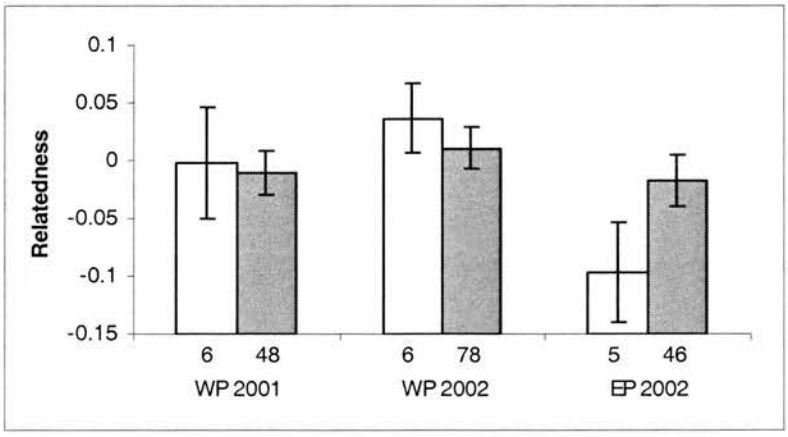


Figure 5.7: Means ± SE of the relatedness of female pairs that were observed interacting (white bars) and those that were not (grey bars) in each focal area for A) pairs within a median of 10 m, B) pairs containing females that were observed for more than one day and C) pairs containing one transient female. Numbers under bars are the number of pairs in each category.

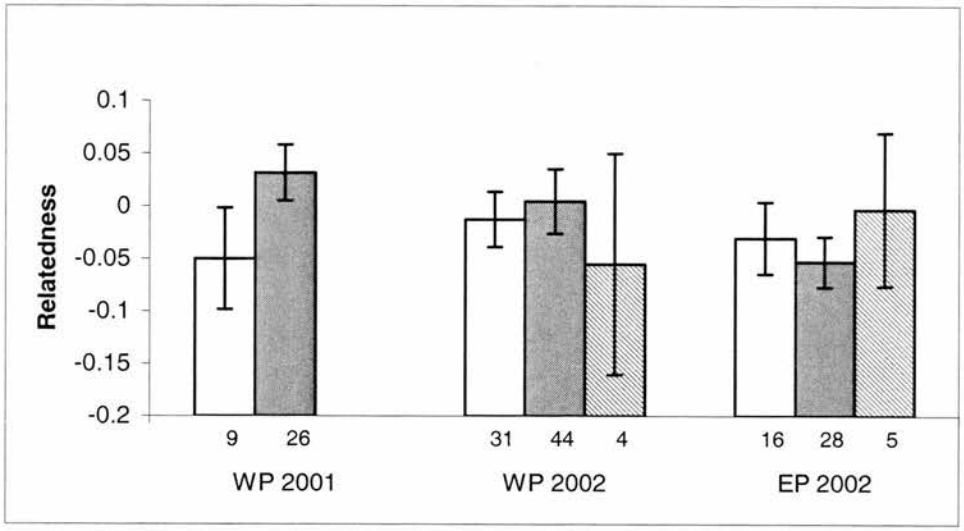


Figure 5.8: Means ± SE of relatedness of pairs that performed low level threats (white bars), high level threats (grey bars) and low level contact (grey diagonal bars) in each focal area. Numbers under bars are the number of pairs in each category.

There was no difference in the distribution of relatedness for pairs where i) both females were present for > 1 day and ii) one female was present for > 1 day and one female was a transient (Kolmogorov-Smirnov two-sample test: WP 2001 $Z = 0.61$, $N_1 = 155$, $N_2 = 54$, $p = 0.808$; WP 2002 $Z = 0.66$, $N_1 = 340$, $N_2 = 84$, $p = 0.747$; EP 2002 $Z = 0.47$, $N_1 = 149$, $N_2 = 51$, $p = 0.960$). Pairs that were observed interacting aggressively were not less related than those that were not, when the two categories of pairs described above were tested separately (Figure 5.7 B,C; Mann-Whitney U tests: i) WP 2001 $Z = -1.09$, $p = 0.279$; WP 2002 $Z = -0.13$ $p = 0.894$; EP 2002 $Z = -0.63$, $p = 0.534$; ii) WP 2001 $Z = -0.30$ $p = 0.778$; WP 2002 $Z = -0.68$, $p = 0.498$, EP 2002 $Z = -1.20$, $p = 0.243$).

There was no significant correlation between the relatedness and the median distance separating a pair or between the relatedness and the number of hours a pair was observed together in the focal area (Table 5.6).

Table 5.6: Spearman's correlations between the relatedness and both the median distance between a pair and the number of hours a pair was observed together in the focal area. No correlations were significant at $p < 0.05$.

Area	Year	Median distance	Number of hours
WP	2001	0.018	-0.077
WP	2002	0.032	-0.088
EP	2002	0.097	-0.008

There was no indication that females interacted more with unrelated then related neighbours (Sign test: WP 2001 negative = 8, positive = 9, p = 1.00; WP 2002 negative = 13, positive = 18, p = 0.472, EP 2002 negative = 11, positive = 13, p = 0.839). Sample sizes are smaller than those used for the GLM (see below) because females with no interactions over the season could not be included.

5.4.4 Analyses of individual interaction rates

Heterozygosity ranged from 0.45 – 1.00 for females in each focal area (Figure 5.9) and did not differ significantly from the normal distribution when years were considered separately (Kolmorogov- Smirnov goodness-of-fit test, $p > 0.05$ for all focal areas). There was no difference between the heterozygosity of females in different focal areas or years (one way ANOVA: $F_{2,93} = 0.32$, $p = 0.726$). Heterozygosity was not correlated with mean relatedness in 2001 (Pearson's correlations $r = -0.013$ $n = 29$, $p = 0.947$) but was weakly negatively correlated in 2002 (Pearson's correlations $r = -0.247$, $n = 67$, $p = 0.044$).

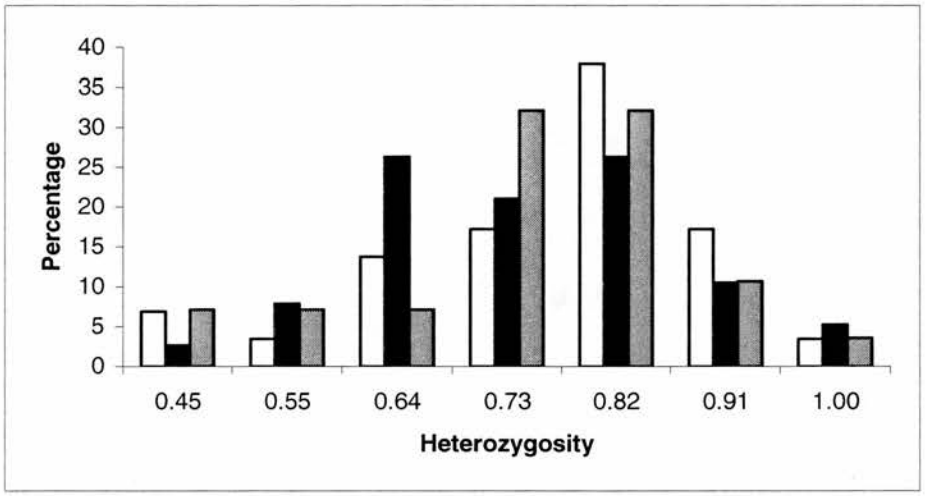


Figure 5.9: Distribution of heterozygosity estimates in WP 2001 (white bars), WP 2002 (black bars) and EP 2002 (grey bars).

The number of aggressive interactions per hour for individual females included in the GLMs of aggression rate ranged from 0 to 0.31 in 2001 and from 0 to 2.0 in 2002 (Figure 5.10). Different main effect variables and interaction terms were retained in the 2001 and 2002 models of interaction rate (Table 5.7). Fewer variables were retained in 2001 and they explained more of the variance than in 2002 (2001 $r^2_{(adj)} = 0.615$; 2002 $r^2_{(adj)} = 0.244$). In 2001, the only interaction term retained in the model was between

relatedness and density of neighbours (Table 5.7). Females with high relatedness to neighbours had high rates of aggressive interactions, especially at low density (Figure 5.11A). However, when females had low relatedness to neighbours, interaction rates increased with density. In 2002, the only interaction term retained in the model was between relatedness and the distance to the nearest pool (Table 5.7). When females had high relatedness to neighbours the rate of initiated aggressive interactions increased further from pools (Figure 5.11B). Conversely, when females had low relatedness to neighbours, interaction rates decreased further from pools.

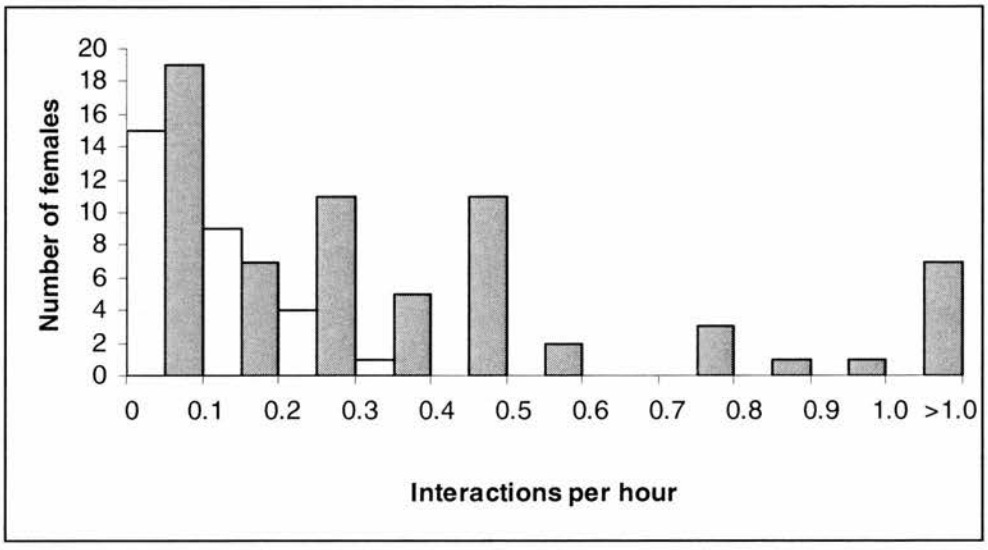
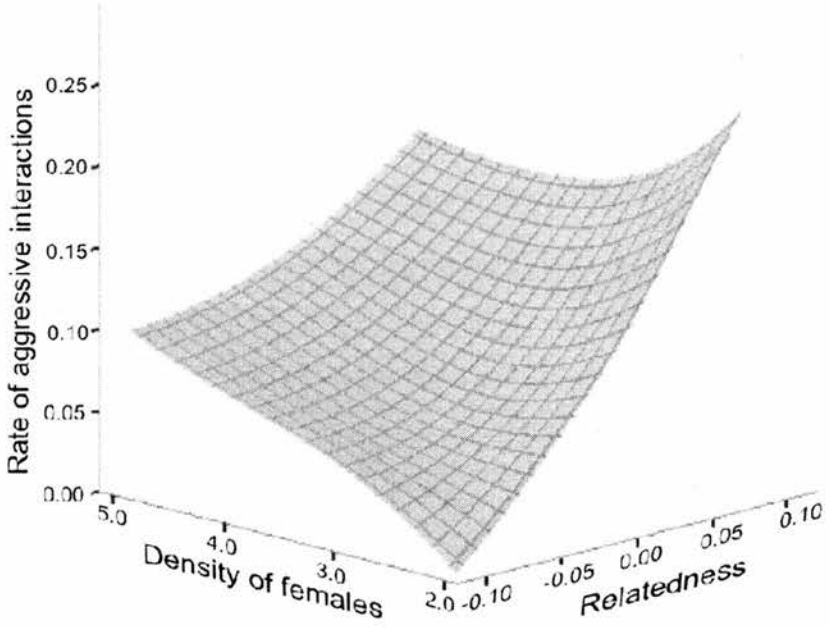


Figure 5.10: Histogram of aggressive interaction rates of individual females included in the general linear model of aggression rate in 2001 (white bars) and 2002 (grey bars).

Table 5.7: Results of the general linear model of aggressive interaction rates per female in 2001 ($F_{5,23}$ = 9.961, $p < 0.001$) and 2002 ($F_{8,58}$ = 3.667, $p = 0.002$). Only terms retained in the models are reported. Significant results are highlighted in bold.

Variable	2001			2002		
	Coefficient	F	p	Coefficient	F	p
Relatedness	**2.350**	**12.166**	**0.002**	**-1.079**	**4.924**	**0.030**
Heterozygosity	----	----	----	0.252	3.836	0.055
Density	0.012	0.998	0.328	----	----	----
Distance to pools	**-0.011**	**15.421**	**<0.001**	0.005	0.765	0.385
Movement	----	----	----	**0.086**	**13.574**	**0.001**
First day	----	----	----	0.004	3.451	0.068
Resident / non-resident	**0.093**	**12.132**	**0.002**	-0.092	3.224	0.078
Focal area	----	----	----	**-0.080**	**5.211**	**0.026**
Relatedness x density	**-0.543**	**5.954**	**0.023**	----	----	----
Relatedness x distance to pools	----	----	----	**0.197**	**5.108**	**0.028**

A



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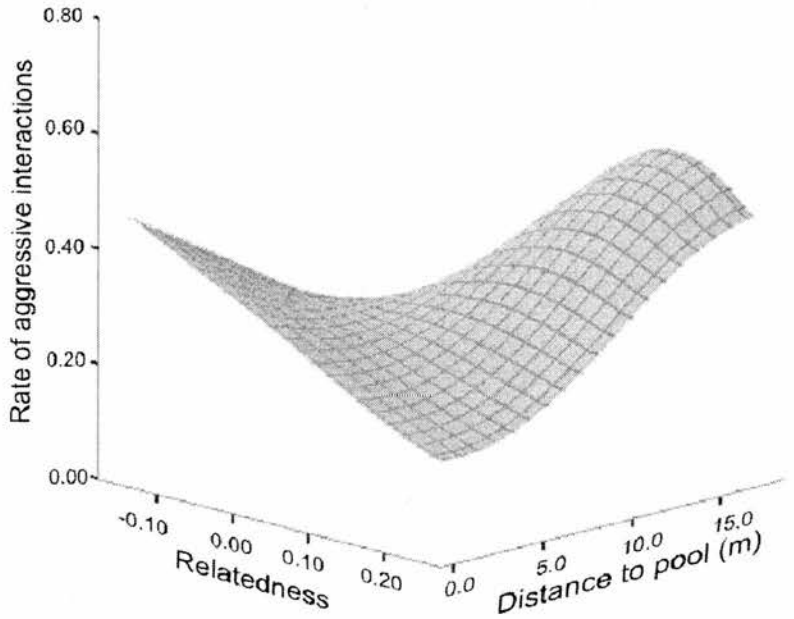

Figure 5.11: Smoothed plots of predicted values of rates of aggressive interactions from the general linear model in A) 2001, plotted against relatedness and density of females and B) 2002, plotted against relatedness and distance to the nearest pool. Plots were rotated to display the clearest and least obscured surface. Data points (not shown) were fairly evenly distributed across the graph in 2001. However, in 2002, there were few females with relatedness vales > 0.10 or that were > 15 m away from a pool.

In 2002 females with higher heterozygosity tended to have higher rates of aggressive interactions than those with lower heterozygosity, although this was not quite significant (Table 5.7). Heterozygosity was not retained in the model in 2001 (inclusion of heterozygosity in the final model yielded the following result: $F_{1,23} = 0.55$, $p = 0.465$).

The 2001 model also indicated that rates of aggressive interactions significantly increased as the distance to the nearest pool decreased (Table 5.7). This is consistent with the results from 2002 for females with low relatedness to neighbours only (Figure 5.11B). Residents also had a significantly higher rate of initiated interactions than non-residents in 2001 (Table 5.7; Figure 5.12). However, in 2002, non-residents tended to have a higher rate of initiated interactions (Figure 5.12), although this was not significant (Table 5.7).

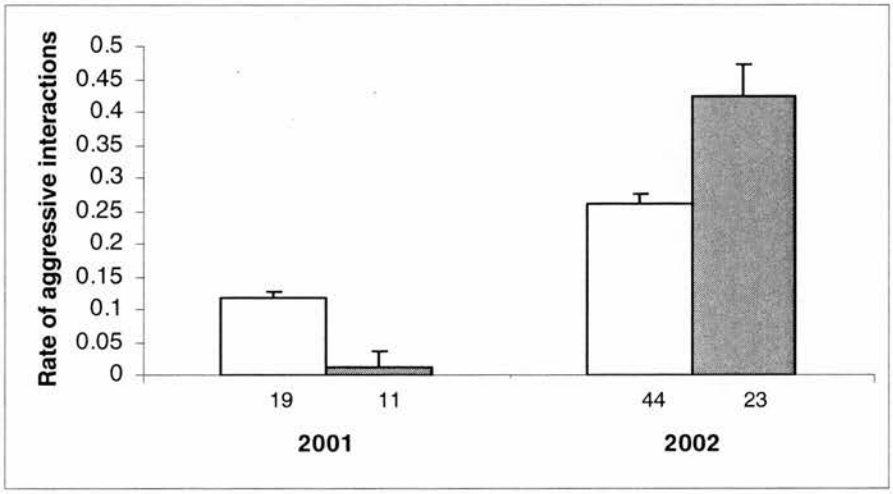

Figure 5.12: Fitted means ± SE of residents (white bars) and non-residents (grey bars) in 2001 (WP only) and 2002 (WP and EP). Numbers of females in each category are indicated under bars.

There were several variables in the 2002 model that were not retained in 2001. Females that moved around the focal areas more in 2002 had a significantly higher interaction rate. Females arriving later in the season also tended to have higher interaction rates. Finally, females in the EP had significantly higher interaction rates (fitted mean: 0.41 ± 0.03 SE) that those in the WP (fitted mean: 0.27 ± 0.03 SE). The only main effect variable not retained in 2002 was density. However, similar to the pup stage model, density co-varied with focal area (t-test: $t = -4.39$, d.f. = 65, $p < 0.001$) with the EP having a higher mean density of females that the WP (EP 7.73 ± 0.51 SE; WP 5.06 ±

0.37 SE). Thus, the focal area with the higher density contained females with higher interaction rates. If focal area was replaced by density in the model, it explained a significant amount of variation ($F_{1,58}$ = 4.09, p = 0.048), although less than focal area (Table 5.7).

Variables were compared between years to investigate whether different conditions in 2001 and 2002 may have caused the differences between the two models. Females in 2002 had significantly higher densities, had their first day in the focal areas earlier, and moved less (Table 5.8). There was no difference between years for mean relatedness, heterozygosity or the distance to the nearest pool. There was also no association between the year and the number of females included in the model that were categorised as resident or non-resident (χ^2 = 0.01, d.f. = 1, p > 0.05). Significant differences between years may have been due to the inclusion of the EP in 2002 and not in 2001. However, when only the data from the WP in 2002 was used, there were still significant differences between years in the density (t = 3.84, d.f. = 66, p < 0.001), movement (t = 3.20, d.f. = 66, p = 0.002) and first day in the focal area (t = 3.01, d.f. = 66, p = 0.004).

Table 5.8: Comparison of continuous explanatory variables in 2001 and 2002 using t-tests (d.f. = 95). Distance to pools was transformed using log(x+1) to achieve normality. Back transformed means and SE are reported.

Variable	2001		2002		t	p
	Mean	SE	Mean	SE		
Relatedness	-0.01	0.01	-0.01	0.01	0.01	0.995
Heterozygosity	0.76	0.02	0.74	0.02	0.74	0.463
Density	3.34	0.16	6.18	0.34	5.34	<0.001
Distance to pools	3.49	0.09	4.52	0.08	1.55	0.125
Movement	1.56	0.20	0.76	0.09	4.19	<0.001
First day	50.48	1.54	41.24	1.04	4.94	<0.001

5.5 Discussion

Both kinship and genetic diversity have been identified as factors that can affect the social behaviour of individuals with a population (Holmes 1988; Waldman 1988; Eklund 1996; Tiira et al. 2003). However, few studies have focussed on whether these factors affect aggressive interactions of pinnipeds (but see Hanggi & Schusterman 1990), with most studies of kinship concentrating on spatial patterns of relatedness and fostering behaviour (e.g. Perry et al. 1998; Schaeff et al. 1999; Pomeroy et al. 2001; Gemmel 2003) and those on genetic diversity assessing its effect on life history traits or

disease resistance (e.g. Coltman et al. 1998; Amos et al. 2001a; Acevedo-Whitehouse et al. 2003; Bean et al. 2004). This study provides some of the first indications that the behavioural interactions between female grey seals on the breeding colony may be modified by both their relatedness to other females and their heterozygosity.

Rates of aggression between females were low and most observed interactions consisted of threatening behaviour only (Table 5.3; 5.4), which is similar to previous studies of grey seal colonies in the UK (Anderson & Harwood 1985; Kovacs 1987; Redman 2002) and Canada (Boness et al. 1982; Haller et al. 1996). Redman (2002) previously reported seasonal rates of aggression ranging from 0.11 to 0.17 on North Rona. The seasonal rate of aggression estimated for 2001 fell within this range but the 2002 estimates were higher than previously reported (Table 5.3). Daily rates of aggression were also significantly higher in 2002 than 2001 but similar in the two focal areas (Figure 5.4). Additionally, there were significant differences in the frequency of the different intensities of interactions between years, mainly due to variation in observed frequency of high and low level threats (Table 5.4). While this may represent real differences in threatening behaviour it is also possible that slight unintentional inconsistencies may have occurred in recording of intensity of interactions between years. However, physical contact during interactions between females was only observed in 2002 (Table 5.4). Thus, rates and intensity of aggression were higher in 2002 than 2001 but were similar between the two focal areas observed in 2002.

Higher levels of aggression in 2002 may be explained by differences in weather patterns. Pools are thought to constitute an important resource for females (Pomeroy et al. 2000b; Twiss et al. 2000a; 2002; Redman et al. 2001) as they are used for bathing and drinking. It was atypically dry at the beginning of the breeding season in 2002 with the scarcity of water leading to fewer, smaller pools than in 2001 (pers. obs.). In warmer, drier conditions, females tend to become more clustered around pools (Twiss et al. 2002). This may explain why female density was higher in the focal areas in 2002 (Table 5.8), as these areas contain some of the largest pools in the Study Area. Thus, a greater local density of females and a scarcity of water are likely to be responsible for the higher rate and intensity of aggression observed in 2002.

Warmer conditions and a shortage of water on the breeding colony has also been found to increase the overall rate of movement by females, including increased locomotion caused by interactions between conspecifics (Redman et al. 2001). Surprisingly, females moved less within the focal areas in 2002 than 2001, when aggression was higher and conditions more stressful (Table 5.8). This discrepancy probably occurred because long-distance movements were not included in this analysis. Instead, the higher density of females in 2002 may have caused movement within the focal areas, by both females and pups, to be restricted. In 2001, females were able to move further within the focal area without encountering other females. This may also explain why movement within the focal area explained a significant amount of variation in the rate of female aggression in 2002 but not in 2001 (Table 5.7).

If most aggression by mothers towards conspecifics occurs to protect pups, mothers with younger, more vulnerable pups should behave more aggressively. Alternatively, females with older pups may be less aggressive because they have become accustomed to their neighbours (Redman 2002). In this study, females with younger pups tended to initiate more aggressive interactions, although not significantly so (Figure 5.5). Females on North Rona with younger pups have previously been found to be more aggressive towards other females (Redman 2002), as have those on Sable Island (Boness et al. 1982). However, maternal aggression was not found to vary with pup stage on the Isle of May (Kovacs 1987; Redman 2002). Redman (2002) suggested the reason for this difference was the greater number of movements made by females (to tidal pools) on the Isle of May. This caused more encounters with unfamiliar females and more aggression to occur close to tidal pools, far from pups, which was unlikely to have functioned in pup protection. Most of the mothers included in the pup stage analysis in this study were from 2002 when a scarcity of water early in the season may also have caused more aggression than usual to be focussed on gaining necessary access to water, regardless of the age of the pup. However, only 36 females were observed for a sufficient length of time to be included, which may have been too few to detect a significant pattern. Additionally, pups vary in the time they remain at each pup stage and thus it is only an approximation of pup age (Redman 2002). If female aggression decreases with pup age, then using pup stage will add further noise into the model.

Immediately before an aggressive interaction, in over half of all recorded cases, no behaviour was observed by either participant making it difficult to make inferences about the purpose of such aggression. However, small movements that were not noticed or visible from the hide may have occurred (e.g. slight changes in posture or movement of whiskers). Boness et al. (1982) reported that an approach by a female preceded most aggression and that the recipient usually moved away after an interaction. While an approach was the most common behaviour recorded before an interaction and moving away was the most common behaviour recorded after, these behaviours were not observed for the majority of interactions (Table 5.5). This may be due to differences between colonies, as the study by Boness et al. (1982) was conducted on Sable Island, or differences in methods. The observations in this study were made on small areas where most females had pups and were in close proximity to each other. Many interactions occurred between females that pupped close together and thus neither was likely to move away and abandon their pups.

As expected, females near pools had higher rates of aggression than those further away in 2001 (Table 5.7). This indicates that pools of water were centres of high rates of aggression in the West Pools in this year, when typical weather patterns were observed. Only females with low relatedness to neighbours were more aggressive near pools in 2002, when water was more limited at the start of the season (Figure 5.11B). Females pupping near water have also been found to be more aggressive than those in poorer quality sites in the Isle of May breeding colony (Twiss et al. 2000a). However, the authors suggest this may reflect differences in densities between sites. In this study, the distance to the nearest pool still explained a significant amount of variation when density was taken into account in 2001 (Table 5.7). Only pools of water that were greater than seal sized (or ~ 0.68 m^2) were used as these were predicted to be areas of high aggression and were easy to identify. However, smaller pools or puddles were also present on the colony, especially near the end of the season when the ground was close to saturation. It may be that these also play a role in female behaviour patterns and constitute an important resource. Focal areas were centred on large pools, but there may also have been pools outside the focal areas that were important to focal females. This could introduce edge effects that were not accounted for. However, in only a few instances were focal females observed using pools outside the focal areas, suggesting this did not strongly influence results.

The relationship between aggression rate and the distance to pools was more complex in 2002. Close to pools, aggression levels were higher, but only for females with low relatedness to neighbours (Figure 5.11B). Conversely, females with high relatedness to neighbours were less aggressive near pools (Figure 5.11B). It is possible that this is an example of females modifying their behaviour based on kinship. Females may be more tolerant of relatives sharing pools and thus initiate fewer aggressive interactions. Reducing competition and allowing kin to pup near a pool could increase the recipient's fitness, by lowering the likelihood of mother-pup separation, and thus the indirect fitness of the donor. Equally, those females with low relatedness to their neighbours may not be willing to share pools and may have to initiate a high number of interactions in order to compete for this resource.

As the distance from pools increased though, the relationship was reversed so that the highest aggression was by females with high relatedness to neighbours (Figure 5.11B). Away from the immediate vicinity of pools, more aggression by females is probably directed at protecting their pups. Here there are less obvious benefits to females that bias behaviour towards relatives. Therefore, aggression rate may not be expected to differ with relatedness to neighbours far from pools. The apparent finding that females with higher relatedness to neighbours initiate more interactions than those with lower relatedness to neighbours is difficult to explain.

Results from 2001 also do not support the idea that females with higher relatedness to neighbours have lower rates of aggression. In fact, in that year, females with high relatedness to neighbours appeared to have higher rates of aggression, especially at low densities (Table 5.7; Figure 5.11A). There does not appear to be an obvious biological explanation for this behaviour. However, the relatedness term in the model only indicates the average relatedness to neighbours, not which individuals a female interacted with. When the relatedness of interactants was directly compared to those that were not observed interacting, using pairwise estimates (Figure 5.4) and estimates for individual females, there was no evidence of reduced aggression between relatives. Nevertheless, in these analyses other variables that may affect aggression rate were not taken into account, such as those included in the GLM. Thus, results are contradictory

and inconclusive but suggest that kin selection for reduced female-female aggression, if present, is a very weak force.

There are several reasons why kin-biased behaviours between females may be unlikely to occur in the breeding colony. As mentioned above, differential treatment of kin is only likely if there is competition between females and is less likely if aggression is directed at protecting pups. (It is also possible, though, that females may bias aggression towards unrelated pups and thus more aggression may occur between unrelated females for the purpose of pup protection). For kin selection to occur in a population, one prerequisite is that interactions occur between relatives. On North Rona, breeding females may be unable to bias behaviour towards kin because so few neighbours are close relatives (Figure 5.6). A lack of close relatives within the focal areas was not unexpected, as kin clustering was not found in the Study Area (see Chapter 3). Furthermore, there was no evidence that females preferred to pup near their relatives, as females did not tend to preferentially interact with transients they were less related to (Figure 5.7C). Kin-biased behaviours require that the relatedness of interactants be above some threshold in order that $rb - c > 0$. For example, dominant female Japanese macaques *Macaca fuscata* are more tolerant of subordinates feeding when kinship is 0.25 or greater, but do not bias behaviour towards females with a relatedness of 0.125 (Belisle & Chapais 2001). For breeding female grey seals, the threshold for the relatedness value would have to be low for kin-biased behaviour to occur at any observable frequency.

For kin selection to act, kin must be able to distinguish each other from non-kin. As females only have one pup a year, kin (other than mother-daughter pairs) would have to recognise each other through phenotype matching (i.e. comparing individuals to themselves or their mothers) and are unlikely to use familiarity. Mothers sniff their pups repeatedly after birth and before suckling (Fogden 1971; Burton et al. 1975) although vocal cues may also be used to identify pups at some colonies (McCulloch & Boness 2000). Adult females have also been observed sniffing each other on the breeding colony. Consequently, odour cues, which have been implicated in recognition systems in other mammals (Holmes 1988; Mateo 2002), appear the most likely recognition mechanism in grey seals.

The presence of kin recognition abilities in a species can be determined through kin discrimination tests or through observed differential behaviour of kin compared to non-kin (Komdeur & Hatchwell 1999). In pinnipeds, fostering (or tolerance of milk stealing) is one behaviour where there appears to be an obvious advantage to kin-biased behaviour. In one species of Otariid, the Antarctic fur seal *Arctocephalus gazella*, females were found to be more closely related to their foster pups than average, suggesting some form of kin recognition (Gemmell 2003). However, in phocids, including harbour seals *Phoca vitulina* and grey seals, fostering females do not differentiate between related and unrelated pups, even on colonies where levels of fostering are as high as 28% (Perry et al. 1998; Schaeff et al. 1999). Moreover, as indicated by the presence of fostering and milk stealing, females can make recognition errors as to the identity of their own pup, especially on crowded, disturbed breeding colonies (Fogden 1971; Perry et al. 1998; McCulloch et al. 1999). This suggests that mothers may have poor kin recognition abilities. It is possible, however, that adults may use cues to recognise relatives that are not available for pups (Roulin & Hager 2003). For instance, communally breeding rodents cannot discriminate between their own and unrelated pups (Holmes & Sherman 1982) but are good at identifying adult kin (Arcarco & Eklund 1999; Mateo 2002).

Another important finding of this study was that females with higher heterozygosity tended to be more aggressive, although this trend was only observed in 2002 (Table 5.7). This could indicate that females with greater genetic diversity are better at protecting their pups from other potentially threatening females. Although genetic diversity has been positively correlated with reproductive success in grey seals on North Rona, reproductive success was measured only in terms of whether a female's pup was sampled in a given year (Amos et al. 2001a). An analysis of the effect of maternal genetic diversity on pup survival or mass at weaning would indicate whether females with greater genetic diversity are actually better mothers.

The positive (almost significant) correlation between heterozygosity and aggression rate may also indicate that females with greater genetic diversity have greater competitive ability. Specifically, these females may initiate aggressive interactions at a higher rate to access and defend better quality pupping sites. If females were more aggressive because they were better competitors, females with higher heterozygosity should cluster around

water. This is supported by a negative correlation between the distance to the nearest pool of water and heterozygosity in 2002 (Pearson's correlations $r = -0.276$, $n = 67$, $p = 0.024$) but not in 2001 ($r = 0.199$, $n = 29$, $p = 0.302$). However, analysis of the entire Study Area in both years suggests that there is no relationship between genetic diversity and distance from pupping sites to pools of water (Table 4.7). Conversely, mothers in the Study Area in 2002 with higher genetic diversity pupped in areas of greater female density, which may represent areas of better habitat (Chapter 4).

The negative effects of inbreeding and lower genetic diversity are typically greater in more stressful situations (Audo & Diehl 1995; Crnokrak & Roff 1999; Meagher et al. 2000; Reed et al. 2002). This may explain why there was no relationship between heterozygosity and aggressiveness in 2001. Densities were lower and pools more abundant in the focal area in this year, which could have decreased competition and stress. Additionally, an epizootic of the phocine distemper virus (PDV) occurred in Europe in 2002 among harbour seals (Jensen et al. 2002), in which the disease is fatal (reviewed in Heide-Jørgensen et al. 1992). This virus infects grey seals but they suffer little mortality and its effects are believed to be minimal (reviewed in Heide-Jørgensen et al. 1992). Nearly all the females on North Rona showed evidence of recent exposure to PDV and some individuals tested positive for the virus (Pomeroy et al. submitted). Individuals with lower genetic diversity may be less immunologically competent in fighting infections (Ferguson & Drahushchak 1990; Coltman et al. 1999; Acevedo-Whitehouse et al. 2003). Therefore females with low heterozygosity may have been most affected by the virus and thus less able to protect their pups. However, the effects of PDV on grey seal behaviour are unknown. Since aggression rates were higher in 2002 than in 2001, before the epidemic occurred, this hypothesis seems unlikely.

The strength of correlations between microsatellite-based measures of heterozygosity and fitness traits is usually weak (Coltman & Slate 2003). In 2001, the smaller sample size of 29 females (compared to 67 females in 2002) may not have been sufficient to detect a relationship between aggression rate and heterozygosity. Indeed, the power of this variable to explain aggression rate in the GLM was less in 2001 (Beta score = 0.110) than in 2002 (Beta score = 0.487). Therefore, the chance of finding a significant relationship between aggression rate and heterozygosity in the model, if it was present, was only approximately a 10% in 2001 compared with almost a 50% in 2002. However,

it is also possible that the result from 2002 was close to significance purely by chance and does not represent a biologically relevant relationship between behaviour and genetic diversity.

The GLMs investigating factors affecting individual female aggression used a rate as the response variable. Rate or ratio data has been criticised because dividing by one variable (in this case, hour) does not always remove the effects on the other (number of aggressive interactions) and thus results may be biased and unreliable (Packard & Boardman 1999). However, an increase in one hour should cause a consistent increase in the number of interactions. Indeed, there was no correlation between the number of hours observed and the rate of aggression in either year (Spearman's correlations: 2001 $r_s = 0.125$, n = 29, p = 0.520, 2002 $r_s = -0.027$, n = 67, p = 0.830) suggesting that the data had been appropriately adjusted for the number of hours observed. In addition, when residuals from a linear regression of the number of initiated aggressive interactions (transformed using $\log10(x+1)$ to achieve constant variance) on the number of hours observed were used, results from the models were similar.

The amount of variance explained by the models was relatively low, especially in 2002. This suggests that there are other factors that affect aggression that were not included in the model, or that the time scale of the model was not fine enough. For each female, variables were averaged over the season. While conditions for some females remained relatively constant over their observation period, for others conditions changed on a daily or hourly basis. For instance, some females spent most of their time far from pools but occasionally travelled to a pool where most of their interactions occurred. In order to account for this behaviour, aggression would need to be modelled on an hourly basis.

Some unexplained variation may also be due to differences in the temperament or personality of individual females. In many vertebrate species, individuals consistently vary in the expression of behaviours, including aggression (Réale et al. 2000; de Boer et al. 2003). Additionally, individuals may show consistent differences across behaviours, such that aggressive individuals tend to be bolder and display more exploratory behaviour (Wilson et al. 1994). Unfortunately, not enough females were observed in two years (n = 5 for females observed for more than one day in both years) to determine whether the aggressiveness of individual female grey seals varies consistently between

years. In future, it would also be interesting to examine whether aggression co-varies with other behavioural traits in this population.

The models explaining aggression rate were inconsistent between 2001 and 2002 (Table 5.7), and results for relatedness to neighbours, heterozygosity and the distance to pools have been discussed. In both years, the rate of aggression generally increased with density although in 2001 this relationship was confounded by an interaction term with relatedness (Figure 5.11A). Other variables were either not retained in both years or provided conflicting results. For example, in 2001 residents had higher interaction rates while in 2002 non-residents tended to have higher interaction rates (Figure 5.12). This may be because females were more likely to enter the focal area in search of water in 2002 and be involved in direct competition for access to pools. Conversely, most non-resident females in 2001 may have been looking for a suitable pupping site only and be less likely to initiate interactions. Indeed, some of the highest interaction rates by non-residents in 2002 were those females that entered the focal areas for a short period of time on several occasions near the start of the season, when conditions were driest, and spent time in pools of water.

In conclusion, females in the North Rona breeding colony were found to display low levels of intrasexual aggression. The majority of evidence suggests that this aggression was not moderated by relatedness to other females. In general, females that were near pools and in crowded conditions were more aggressive. However, results were inconsistent between the two years studied, which may have been due to differences in environmental conditions. Females with higher heterozygosity tended to be more aggressive in the year when conditions were more stressful. This suggests they may be better mothers and the more dominant females in the colony.

Chapter 6: Mate choice and patterns of male reproductive success in a grey seal *Halichoerus grypus* colony

6.1 Introduction

Recent advances in molecular techniques have helped to achieve a better understanding of mating systems and individual reproductive success. In many animal species, paternity analysis has revealed how behavioural measures of reproductive success relate to true patterns of parentage (Birkhead & Møller 1993; Petrie & Kempenaers 1998). Much of this work has focussed on behaviourally monogamous birds, where extra-pair fertilisations have been found to form a significant part of the mating system in some species (reviewed in Griffith et al. 2002). Paternity analysis has also helped to elucidate patterns of reproductive success in several mammalian species, including pinnipeds (Pemberton et al. 1992; Coltman et al. 1998; Hoelzel et al. 1999; Worthington Wilmer et al. 1999; Hoffman et al. 2003; Baker et al. 2004).

Behavioural observations suggest most species of terrestrially breeding pinnipeds show a moderate to high level of polygyny (reviewed in Le Boeuf 1991). Males do not contribute to parental care and thus maximise reproductive success by mating with as many females as possible. As females are spatially and temporally clustered in breeding colonies, a few dominant males can defend resources, or access to females, thereby gaining a disproportionate number of matings (Emlen & Oring 1977). Genetic determination of paternity has enabled a direct comparison of behavioural mating success of dominant males and their realised reproductive success. For example, behavioural measures of reproductive success of individual southern elephant seal males *Mirounga leonina* generally agreed with those estimated using genetic paternity assignment (Hoelzel et al. 1999; Fabiani et al. 2004).

The grey seal mating system has been studied intensively using both behavioural observations (Anderson et al. 1975; Boness & James 1979; Anderson & Harwood 1985; Godsell 1991; Twiss et al. 1994; 1998; Tinker et al. 1995) and molecular data (Amos et al. 1993; 1995; 2001b; Ambs et al. 1999; Worthington Wilmer et al. 1999; 2000; Lidgard et al. 2004). Behavioural observations indicate that grey seals have a moderately polygynous mating system (Anderson et al. 1975; Boness & James 1979; Tinker et al. 1995; Twiss et al. 1998). Males come ashore after the start of the breeding season and compete with each other for access to females, forming an approximate

dominance hierarchy (Anderson et al. 1975; Boness & James 1979; Twiss et al. 1998). Dominant males do not have fixed territories but defend a position around a group of females (Anderson et al. 1975; Boness & James 1979; Twiss et al. 1994). Those males with the greatest behavioural dominance typically stay ashore the longest and have the greatest observed mating success (Anderson et al. 1975; Godsell 1991; Twiss et al. 1994; Tinker et al. 1995; Lidgard et al. 2001). Conversely, subordinate males occupy peripheral areas of the colony and try to obtain matings using alternative strategies. These strategies may be adopted by smaller, younger males and generally result in lower observed mating success (Anderson & Fedak 1985; Godsell 1991; Twiss et al. 1994; Lidgard et al. 2001).

Females come into oestrus near the end of lactation (Anderson et al. 1975; Boness & James 1979; Pomeroy 1999). During this time, females are frequently aggressive towards approaching males, including those they eventually mate with (Anderson et al. 1975; Anderson & Harwood 1985). Some of this aggression may take place to incite competition between males, which could maximise a female's chance of mating with a high quality male (Anderson et al. 1975; Boness et al. 1982). Earlier in lactation, aggression by females towards males may function as a pup protection mechanism as well (Boness et al. 1982; Maestripieri 1991). Male harassment can reduce maternal performance and may promote clustering of females within pinniped colonies (Boness et al. 1995; Cassinni 2000).

Paternity analysis in the grey seal also suggests a moderately polygynous mating system, but the proportion of parentage assigned to dominant males was not as great as expected from behavioural data (Amos et al. 1993; Ambs et al. 1999; Worthington Wilmer et al. 1999; Lidgard et al. 2004). For example, 43% of offspring of females on Sable Island were not sired by the male predicted from observations (Ambs et al. 1999). Subordinate males on the periphery of the colony were also found to father some pups, but a smaller proportion each than dominant males (Worthington Wilmer et al. 1999; Lidgard et al. 2004). Overall, the molecular data suggest that only a handful of males within a colony are highly successful, with most pups being sired by different males that have comparatively low reproductive success (Worthington Wilmer et al. 2000).

Worthington Wilmer et al. (1999) found that many pups could not be assigned fathers in two UK colonies, despite large numbers of males, including most dominant males, being sampled. This may be for several reasons. First, females do not always breed every year and those not returning to their usual breeding colony may mate elsewhere (Pomeroy et al. 1999). This has been found to be the case in Antarctic fur seals *Arctocephalus gazella* (Hoffman et al. 2003). Second, while a reasonable proportion of males were sampled in some regions in some years (~ 80%; Worthington Wilmer et al. 1999), complete sampling of entire colonies over many years was not feasible. This is because subordinate males occur on the periphery of the colony or in access gullies, some only for a brief period of time, and are thus very difficult to sample. The effect of this incomplete sampling will depend on the spatial and temporal occurrence of males. Finally, Worthington Wilmer et al. (1999) speculated that many males were not sampled because they adopted the alternative strategy of mating in the water, rather than within the colony.

Further analysis of grey seal paternities has suggested the possibility of individual mate choice (Amos et al. 1995; 2001b). The traditional view of mate choice is that mates are chosen on the basis of specific traits, such as size or dominance, that indicate individual quality (Qvarnström & Forsgren 1998). In its simplest form, this model predicts all individuals will choose the same 'best' mate (Andersson 1994). However, individuals may also choose mates based on genetic compatibility in order to produce the most genetically diverse offspring (Tregenza & Wedell 2000; Mays & Hill 2004). Thus, each individual would have a preference for a different mate. Choice may occur pre-copulation, if individuals can assess genetic similarity, or post-copulation in polyandrous species that possess an appropriate mechanism (Olsson et al. 1996; 2003; Penn 2002; Foerster et al. 2003; Garner & Schmidt 2003). For example, sand lizards *Lacerta agilis* prefer to associate with genetically dissimilar mates and, post-copulation, females fertilise their eggs with the most genetically dissimilar sperm (Olsson et al. 1996; 2003). The 'best' mate and the most compatible mate strategies may both occur in the same breeding system, but be used at different times or for different types of mates (Mays & Hill 2004).

Mate choice models predict that the sex that invests more in offspring will be the choosiest (Johnstone et al. 1996). Thus, in polygynous systems where males provide

only gametes, females are expected to be the choosier sex and males exhibit little or no choice. However, mating may be more costly to males than previously believed, due to sperm depletion or the cost of courtship behaviour, resulting in some degree of male mate choice (Preston et al. 2001; Wedell et al. 2002; Werner & Lotem 2003). For example, two studies of lekking species (where males provide no parental care) found that males exert mate choice for females they perceive to be the most fecund (Sæther et al. 2001; Werner & Lotem 2003).

Evidence of individual mate choice in grey seals was first uncovered by Amos et al. (1995) who found a larger proportion of full siblings among pups sampled in the North Rona breeding colony than could be explained by male site fidelity alone (estimated at 53 m; Twiss et al. 1994). This suggested individuals were actively choosing the same partner in multiple years. In addition, pups that shared the same mother, but not the same father, were less paternally related than expected (Worthington Wilmer et al. 2000; Amos et al. 2001b). Modelling suggests this was because females mated with males that were more genetically dissimilar from previous partners than expected (but not themselves) (Amos et al. 2001b). However, the possibility of mate choice for genetically compatible mates has not yet been investigated in detail.

6.2 Aims

One aim of this chapter was to examine the possibility of mate choice for genetically dissimilar partners in grey seals breeding in the North Rona colony. Both genetic parents and pairs that were observed mating were predicted to be less related to each other than random. The relatedness of males and females occupying focal areas was also compared to assess whether individuals prefer to associate with genetically dissimilar members of the opposite sex. The possibility of both male and female behavioural mate choice was examined. A second objective of this chapter was to investigate whether observational data could help explain some of the shortfall in paternities found by Worthington Wilmer et al. (1999). More pups were expected to be assigned fathers when sampling effort was greater in the year they were conceived. Males that were observed in the previous year were also predicted to be assigned paternities more often than those that were not. Similarly, females that were absent from the colony the year before they gave birth were predicted to be more likely to have pups that could not be assigned to a sampled male. Paternity assignment was also compared

between regions in the colony. Finally, patterns of aggressive behaviour between males and females were compared between focal areas and years. As female-female aggression was found to be higher in 2002 (Chapter 5), inter-sexual aggression was also expected to be higher in that year.

6.3 Methods

6.3.1 Genetic sampling and relatedness estimates

All individuals used in this chapter were sampled in 2000 to 2003, with the exception of a few females that were sampled in 1998 (n = 6) and 1999 (n = 2). Males and females were usually only sampled in one year, but may have been observed in several breeding seasons (see Chapter 2). Only females that were i) mothers of sampled pups, ii) observed in sexual interactions, or iii) observed in the focal areas, were used in this chapter. Obtaining skin samples of pups can be disruptive when mothers try to protect their pups. Therefore, most pup samples were taken when mothers were captured for the purposes of other studies. The pups of females that were observed having sexual interactions with males the previous year were also specifically targeted in 2002 and 2003. Additional pups were sampled in 2001 from females in the West Pools (WP) focal area and other locations within the Study Area (SA) on the last day of the field season in areas where disturbance was minimal. All sampled pups had mothers with genotypes that matched for at least one allele at each locus (see Chapter 2).

Males were sampled throughout the breeding season whenever the opportunity arose. However, sampling was concentrated specifically in the southern part of SA as this was where many mother-pup pairs were sampled (S. Twiss pers. comm.). The proportion of dominant males that were genotyped each year was calculated as: the number of genotyped males that were observed for more than one day / the total number of males that were observed for more than one day in the colony. Males present for more than one day are considered tenured or dominant males (Boness & James 1979; Anderson & Fedak 1985; Twiss et al. 1994). The vast majority of observed genotyped males each season were also observed ashore for at least two days (94%). The total number of males observed included all males visible from the hide (all of SA and most of Fianuis South (FS)). All observational data for males were provided by S. Twiss. No data were available for two of the genotyped males, as they were not observed in areas that were visible from the hide.

Samples were genotyped for 11 microsatellite loci, as described in Chapter 2. Relatedness was estimated using RELATEDNESS 5.0 (Queller and Goodnight 1989; Chapter 2). Genotypes from all non-replicate adults sampled between 1997 and 2003 (n = 437) were used to generate background allele frequencies (as in Chapter 5).

6.3.2 Paternity assignment

Paternity analysis was performed using CERVUS 2.0 (Marshall et al. 1998). This programme uses simulations based on population allele frequencies to estimate the likelihood of a male being the father. These simulations require information on the genotyping error rate, the proportion of loci that were typed, the number of candidate males and the proportion sampled. The percentage of loci that were typed incorrectly was estimated at 1.0% per locus (see Chapter 2). The proportion of loci that were typed was determined from the data to be 0.99. Two estimates were used to represent the number of candidate males that may father pups within SA, where the majority of pups in this dataset were sampled. The lower estimate, 136, is the average number of different males that were recorded ashore in SA over the years of the study (S. Twiss, pers. comm.). This is likely to be an underestimate as observations were only made for eight (daylight) hours a day for two-thirds of days during the field season. The upper estimate of the number of candidate males was 450. This is the approximate number of mothers observed pupping during the breeding season in SA and assumes an equal sex ratio of males to females. Paternity analysis was run at two confidence levels: 80% (four out of five assignments correct) and 95% (19 out of 20 assignments correct).

The number of mismatches between genotypes of assigned fathers and pups (once the mother's genotype has been excluded), in relation to confidence levels, can give an indication of how accurately CERVUS is assigning parentage. Mismatches between the pup's genotype and the father's occur in CERVUS because it allows for genotyping errors and mutational events (Marshall et al. 1998). The majority of candidate males that were assigned at 95% confidence should have no mismatches, as the genotyping error rate was only set to 1%. Similarly, pairs with no mismatches should rarely be assigned with less than 80% confidence; this will happen if the father and pup share only common alleles at each locus.

6.3.3 Patterns of parentage

The effect of sampling intensity was examined by comparing the proportion of males sampled each year to the percentage of pups assigned parentage the following year. Males that were observed in the colony in a given year may be more likely to father pups in the following year than those that were not observed. To test this, the number of males assigned paternity that were observed the previous year was compared to the number assigned paternity that were not observed, taking into account the proportion of genotyped males that were observed. Next, the effect of length of stay in the colony on the likelihood of a male fathering at least one sampled pup was investigated. The number of days a male remained in the colony during the observation period each breeding season was compared between males that were and were not assigned paternities, for each year separately. As the duration of observations were similar for all breeding seasons used in this analysis (see results) the data were not standardized for the length of time observed each year.

The proportion of pups that were successfully assigned fathers was compared between the three regions of the colony: SA, FS and Fianuis North (FN) (Figure 3.1). For these analyses, the region pups were born in were used, not the region where their mother was observed in the year they were conceived, because this was unknown for most mother-pup pairs (103/192). However, previous studies have found females are usually faithful to one region of the colony (Pomeroy et al. 1994; in press). Data identifying the regions in which males were observed each year were not available.

The distance between different mothers that had pups with the same father were compared for the i) the year of conception and ii) the year the pup was born. The first analysis will indicate whether males usually mate successfully with females that are in close proximity to each other. The second analysis will indicate how close together, on average, paternal half siblings are born. Analyses were first performed only for paternal half siblings born in the same year, and subsequently for all paternal half siblings. The distance between the birth sites of full siblings (between years) was also determined. Mothers' locations were the pupping locations used in Chapters 3 and 4. Thus, for analyses using the year of conception, pupping location was used as a proxy for position at the time of oestrus.

The ability to assign paternity to pups whose mothers were not observed in the colony in their year of conception was also assessed. Females that were not observed fell into two categories: i) those that first entered into the known female database in the year they gave birth and ii) those that were known but were not observed. All branded females and those tagged before the year they gave birth were assumed to be known females that were identifiable. The proportion of paternities assigned to pups with mothers in the second category (i.e. known but not observed) was compared to those with mothers that were observed in the colony in their year of conception.

To test the prediction that parents were more genetically dissimilar to each other than average, the relatedness of parents was compared to a random sample using a z-test. To create a random sample, the relatedness of all males (n = 59) to all females (n = 378) that were sampled on North Rona was estimated. From this dataset, 1000 relatedness values were randomly selected with replacement using the Resampling Stats Excel add-in, version 3.0 (Simon 1997). These relatedness values (mean = 0.007 ± 0.166 SD) were used throughout this chapter to represent a random sample.

6.3.4 Observed sexual interactions

Sexual interactions were recorded, during *ad libitum* observations, by S. Twiss from 1998 to 2003. The identities of all observed males were determined by S. Twiss. Female identities were determined either from brands or from pelage markings by P. Pomeroy, P. Redman, S. Ruddell or myself. Only those copulations where both the male and female could be assigned identities were used. Two types of sexual interactions were recorded: attempted copulations (the male tried to mount the female but intromission did not occur) and copulations (intromission occurred). Pairs observed in both types of interactions were recorded as copulating. Copulations were further divided into successful (the male completed the copulation) or unsuccessful (the copulation was not completed, often because of disturbance by another male or because the female became agitated).

To test whether the relatedness of pairs observed having sexual interactions was less than expected, the relatedness of genotyped pairs was compared to the random sample using a z-test. This analysis was also performed including only those pairs observed copulating.

Observed sexual interactions were then compared to results from the paternity analysis to estimate how many observed copulations resulted in parentage. The type of interaction and whether or not copulations were successful was compared between those interactions that resulted in parentage and those that did not. Relatedness of pairs observed in sexual interactions that resulted in parentage was compared to the relatedness of pairs observed that were not the parents.

6.3.5 Focal area observations

Observations were made on the WP focal area in two years (2001, 2002), and the East pools (EP) focal area in one year (2002). A full description of the focal areas and the observation regime can be found in Chapter 5. Briefly, continuous observations were made on focal areas for between four and eight hours per day during the breeding season. Observations were made on approximately 60% of days during the field season. Females were identified within and between seasons using pelage markings or, occasionally, brands or flipper tags.

Males were recorded when they entered and left the focal areas. In 2002, this information was recorded on separate data collection sheets, while in 2001 it was recorded along with continuous observations. This change was made to make it easier to identify males from their movements. Each time a male entered the focal area he was given a unique number. At the end of each day, the identity of the males was provided by S. Twiss based on their time and behaviour within the focal area. Occasionally, males could not be given an identity. Males often moved in and out of the focal area and thus any unidentified male was likely to be a male that was observed in the focal area that day. An unidentified male was only included as a 'different' male if he could not have been an identified male (i.e. the locations of all other identified males were known).

If a female was not observed giving birth, the pup's approximate birth date was estimated from its pup stage (Table 3.1). In most instances, females were observed with a stage one pup and thus pupping dates, and also lactation days, were usually accurate to within a day (Table 3.1). Oestrus is thought to occur on approximately lactation day 15 if the lactation period is a standard 18 days (Pomeroy 1999). In order to include all

females that may be in oestrus, taking into account natural variation and inaccuracies in lactation day estimation, oestrus was defined as between lactation days 13 and 17.

In order to investigate whether a female's choice of pupping site was affected by her relatedness to the males present, the relatedness of females to males observed in the focal area at the start of a female's lactation was compared to the random sample using a z-test. Males may also choose to defend areas containing females to which they are not closely related. Therefore, the relatedness of males to females during oestrus was also compared to the random sample.

Continuous observations of all behaviours between females and males within the focal area were recorded on data collection sheets. The following information was recorded for all male-female aggressive interactions: time, date, identity of initiator and identity of recipient. An interaction was recorded as aggressive if at least one participant performed an aggressive behaviour including: open mouth threat (mouth open, whiskers erect towards the recipient), lunging towards the recipient, flippering (rapid movement of the fore-flipper), biting or chasing. Following Redman (2002), if an interaction stopped and started again, one bout was recorded if there was less than one minute between interactions and there was no obvious outcome (e.g. one participant moving away). All attempted copulations and copulations were preceded by aggression and these were recorded along with the aggressive interactions.

Seasonal interaction rates (per hour, per female) for each area and year were calculated as: total number of aggressive interactions observed / (median number of females observed per day x total number of hours observed), following Redman (2002). The number of interactions per day was also compared between the WP in 2001 and 2002 and between the WP and the EP in 2002 using general linear models. The number of females observed per day, the number of males observed per day and the number of hours observed per day when males were present were included as covariates. Days were only included if at least one male was observed in the focal area. The number of interactions was transformed with $\log_{10}(x + 1)$ to achieve normality. Residuals from the model were tested for normality using Kolmogorov-Smirnov tests. Levene's test of equality of error variances was used to test for homogeneity of variance.

Data from focal areas and years were combined for all further analyses due to low sample sizes. Records of copulations observed during the normal focal area observation period were supplemented by those observed within the focal area outside the observation time. These records formed part of the larger data set of observed copulations used in the previous section. This only occurred in 2002 when each focal area was observed for half a day (either morning or afternoon).

The distribution of aggressive interactions over the lactation period was determined to investigate whether the number of inter-sexual aggressive interactions increased during oestrus. The number of aggressive interactions per female observed on each day of lactation was calculated for each lactation day up to day 18. Aggression was then compared between days during oestrus (lactation day 13 – 17) and days earlier in lactation (lactation day 1 – 12).

The next two analyses tested whether i) given the males that were present, focal females chose to mate with the males they were least related to and ii) given the choice of females that were in oestrus, males chose to mate with the focal females they were least related to. In the first analysis, for females, the relatedness to males that they were observed having sexual interactions with was compared to those that they were not. Only females that were observed having sexual interactions and only males that were observed in the focal area when the female was in oestrus were included. In the second analysis, for males, the relatedness to females (that were in oestrus) that they were observed having sexual interactions with was compared to those they were not. In addition, for those pairs observed having aggressive interactions while the female was in oestrus, the relatedness of pairs also observed having sexual interactions was compared to those that were not.

Finally, patterns of parentage were investigated for pups of focal females. For each year and focal area, parentage was examined for pups that were born and conceived in the focal areas. Parentage was compared between pups sampled in the same location and year.

Statistical analyses were performed in SPSS 11.0 unless otherwise stated. Since the relatedness of pairs was often not independent (i.e. some individuals formed part of

more than one pair), for analyses comparing relatedness values between two sets of data, significance was tested using 10 000 Monte Carlo simulations by repeat sampling from the data. All relatedness estimates are presented as means ± standard deviation.

6.4 Results

6.4.1 Paternity analysis

A total of 192 mother-pup pairs and 59 males were genotyped (Table 6.1). Mother-pup pairs consisted of 134 different mothers, as some mothers had pups sampled in multiple years (one pup n = 98; two pups n = 19; three pups n = 12; four pups n = 5). As approximately 1000 pups are born annually on North Rona (C. Duck pers. comm.), sampled pups represent only a small proportion of all pups born on the island. Individual males were often observed in more than one year (one year n = 12; two years n = 19; three years n = 8; four years n = 11; five years n = 7). The proportion of dominant males observed in the colony that were genotyped was greatest in 2002 and lowest in 1999 (Table 6.1). The duration of the observation period also differed between seasons, with a considerably shorter period being observed in 1999 (Table 6.1).

Table 6.1: The number of genotyped mother-pup pairs and males that were observed each year, the proportion of dominant males observed in the colony that were genotyped and the duration of observations.

Year	Number genotyped		Proportion of males genotyped	Duration of observations (days)
	Mother-pup pairs	Males		
1999	0	15	0.22	26
2000	35	31	0.31	43
2001	79	34	0.40	42
2002	50	38	0.51	40
2003	28	35	0.46	41

Paternity analyses suggested that within years, 14% to 58% of sampled pups could be assigned fathers (Table 6.2). When all years were combined, 30% to 48% of sampled pups were assigned a father. As expected, more pups were assigned fathers when the number of potential candidate males was set at 136 than 450. The result using 136 candidate males at the 95% confidence level was identical to that using 450 males at the 80% confidence level. These paternities were used for all further analyses unless otherwise stated.

Table 6.2. The number of paternities and different males assigned by CERVUS for each year when A) 136 candidate males and B) 450 candidate males were used. Results are reported for both 95% and 80% confidence levels (CL). Numbers in brackets indicate the percentage of pups assigned fathers (Paternities) or the percentage of males fathering at least one pup (Males).

		2000	2001	2002	2003	Total
A) 136 candidate males		**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
95% CL	Paternities	5 (14)	34 (43)	27 (54)	8 (29)	74 (39)
	Males	4 (6)	16 (27)	16 (27)	8 (14)	24 (41)
80% CL	Paternities	10 (29)	39 (49)	29 (58)	14 (50)	92 (48)
	Males	8 (14)	17 (29)	18 (31)	13 (22)	31 (53)
B) 450 candidate males						
95% CL	Paternities	5 (14)	23 (29)	23 (46)	6 (21)	57 (30)
	Males	4 (6)	14 (24)	13 (22)	6 (10)	21 (36)
80% CL	Paternities	5 (14)	34 (43)	27 (54)	8 (29)	74 (39)
	Males	4 (6)	16 (27)	16 (27)	8 (14)	24 (41)

There were no mismatches between the genotypes of the assigned father and the pup (when the mother's genotype was taken into account) in 68/74 (92%) cases. There was one mismatch between the genotypes in 5/74 (7%) cases and two mismatches in 1/74 (1%) cases. Nine (out of 118) of the most likely fathers (8%) had no mismatches with the pup but were assigned with less than 95% confidence using 136 candidate males (and less than 80% confidence using 450 males).

6.4.2 Distribution of paternities

Of sampled males, 41% fathered at least one pup over all years (Table 6.2; Figure 6.1). Most males that were fathers sired only one pup each year (Table 6.3). Within a year (2001), the maximum number of pups sired by a male was eight. Over all years, approximately 10% (6/59) of sampled males achieved at least five paternities (Figure 6.1). The same number of these males sired pups in two different years (n = 3) and in three different years (n = 3). Males that achieved no paternities in any year were observed in significantly fewer years (from 1999 to 2002) than those that fathered at least one sampled pup (Mann-Whitney U test, $Z = -3.37$, $N_1 = 33$, $N_2 = 24$, $p = 0.001$). Four of the 35 males achieving no paternities were observed only in 2003 and another three were observed for less than two days in a single year. However, six of the males that were not assigned any paternities were observed in at least three years (when their pups could have been sampled in the following year) for a substantial proportion of the

observation time (mean proportion of time observed for the six males: 0.15, 0.22, 0.29, 0.46, 0.59, 0.81).

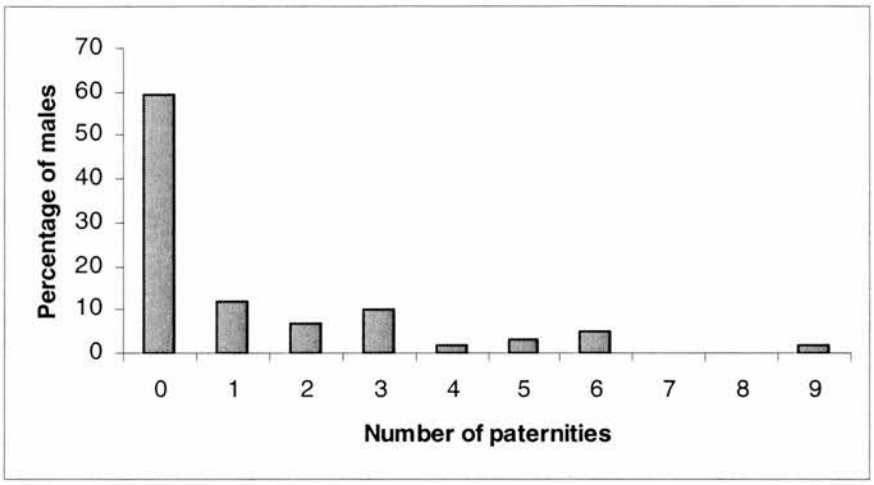

Figure 6.1: The percentage of males that were assigned different numbers of paternities when data from all four years were combined.

Table 6.3: The number of paternities assigned to the 59 genotyped males in each year.

Number of paternities	2000	2001	2002	2003
0	55	43	43	51
1	3	8	10	8
2	1	5	3	0
3	0	1	1	0
4	0	0	2	0
>4	0	2	0	0

Considerably fewer paternities were assigned to pups born in 2000 than in other years (Table 6.2). Fewer males were also genotyped in 1999, when the pups were conceived (Table 6.1) suggesting this may be the cause. However, the most complete sampling of males occurred in 2002, but the percentage of pups assigned fathers in 2003 was lower than in 2001 and 2002. Genotyped males that were observed in the colony in the previous year were significantly more likely to be assigned paternity than those that were not, except in 2000 (Table 6.4). However, the result from 2003 was not significant after Bonferroni corrections for multiple comparisons. The duration of the observation period in 1999 was almost half of that in all other years (Table 6.1), which may explain why fathers assigned to pups in 2000 were not observed.

Table 6.4: The number of males assigned paternity that were and were not observed in the colony the previous year. Numbers in brackets are the number of males expected to be assigned paternity for each category, given the proportion of genotyped males that were observed the previous year. Results of χ^2- tests (with Yates' correction) comparing the observed and expected values are given.

Year	Number of males assigned paternity		χ^2	p
	Observed	Not observed		
2000	1 (1.1)	3 (2.9)	0.01	0.969
2001	16 (8.7)	0 (7.3)	13.43	< 0.001
2002	14 (9.5)	2 (6.5)	5.43	0.011
2003	8 (5.3)	0 (2.7)	4.07	0.023

In 2001 and 2002, males that fathered at least one sampled pup were observed for a significantly greater number of days in the previous season than those that did not (Figure 6.2; t-test, 2001: t = -3.30, d.f. = 29, p = 0.003; 2002: t = -3.99. d.f. = 32, p <0.001). This trend was also observed in 2003, but was not significant (Figure 6.2; t = -1.73, d.f. = 36, p = 0.093). On average, males that fathered a pup remained in the colony for over 25 days, while those that did not typically remained for less than 15 days (Figure 6.2). These analyses only included males that were observed in colony in the previous season. Hence, this could not be performed for the 2000 season as only one male that was observed in the colony in 1999 fathered a pup.

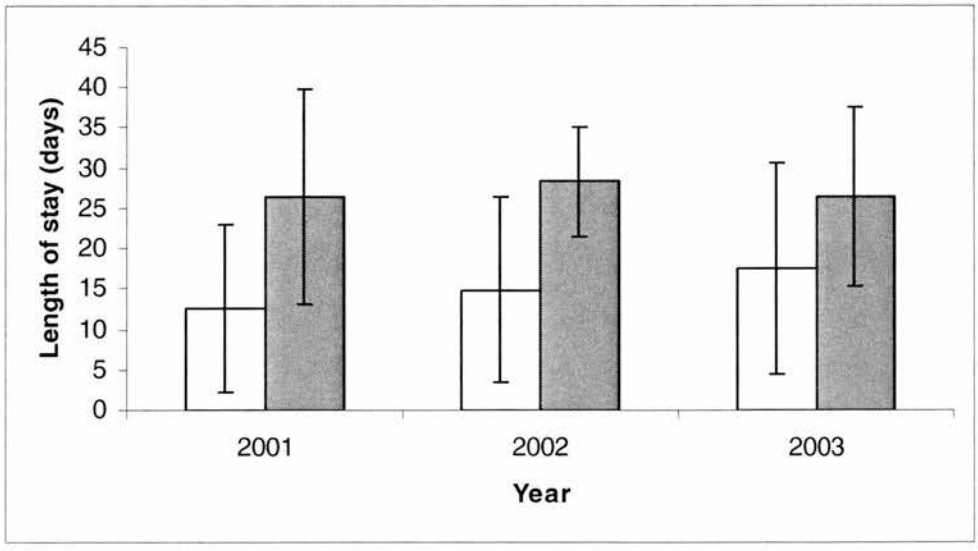

Figure 6.2: Mean (± SD) number of days that males were observed in the previous year, for those males that did not father a pup (white bars), and to those that were assigned at least one paternity (grey bars). The x-axis represents the year pups were born.

Three quarters of all genotyped mother-pup pairs were sampled in SA while only 10% were from FS (Table 6.5). Of the 74 pups that were assigned a father, more than expected were born in FS, while fewer than expected were born in FN ($\chi^2 = 6.12$, d.f. = 2, p = 0.023). Of the 12 pups assigned paternities in FS, half were fathered by the same male.

Table 6.5: The distribution of sampled pups and paternities among regions within the North Rona colony. The '% Total' (under the paternities heading) refers to the percentage of the 74 paternities that were assigned to pups born in each region and sums to 100. The '% pups assigned paternities' refers to the percentage of sampled pups in each region that were assigned paternities and does not sum to 100.

Region	Sampled pups		Paternities		% Pups assigned paternities
	Number	% Total	Number	% Total	
SA	145	75	57	77	39
FS	19	10	12	16	63
FN	28	15	5	7	18

There were four pairs and two sets of three paternal half-siblings born in the same year and whose mothers' locations were known in their year of conception. This resulted in 10 different comparisons. The median distance between mothers of paternal half siblings, in the year of conception, was 36 m (range: 9 – 94 m). Pups with the same father were always conceived in the same region of the colony. When paternal half siblings from all years were considered (n = 29 pups), the median distance between mothers in their year of conception was 41 m (range: 3 – 96 m, n = 31 comparisons). Again, all pups with the same father were conceived in the same region of the colony.

The mother's location in the year the pup was born was known for all paternal halfsiblings born in the same year (Table 6.3). The median distance between mothers of paternal half siblings, in year they gave birth, (n = 45 pups) was 50 m (range: 5 – 190 m, n = 65 comparisons). There was one case of pups with the same father, in the same year, being born in two different regions of the colony (SA and FS). When all years were combined, paternal half siblings (n = 63 pups) were born a median of 54 m apart (range: 4 – 547 m, n = 127 comparisons). Over all years, there were five instances of pups with the same father being born in different regions of the colony; in four cases, the regions were adjacent. In the other, one pup was born in FN and one in SA a year apart.

For mothers with pups sampled in more than one year (n = 36), only three had the same father assigned for two of their pups. An additional mother also had two pups with the same male identified as the most likely father, but with less than 80% confidence for one pup. However, there was only one mismatch between the male's and pup's genotype when the mother's had been taken into account, suggesting that this was likely to be the father. Three of the full siblings were born in consecutive years with the other pair being born two years apart. The location of the mother in the year of conception was known for both years for three of the full sibling pairs. The distances between the mothers of full siblings in the two years of conception were: 25 m, 32 m and 77 m. The distances between birth sites of full siblings in the two years was known for all full sibling pairs and were: 37 m, 46 m, 76 m, and 78 m.

There were only a few known females (n = 15) that were not observed in the year of conception. (Most mothers without locations in the year of conception were identified for the first time in the year the pup was sampled; n = 88). Only two of the pups with known mothers not observed in the year of conception were assigned fathers. Both of these pups were born in FN; one mother could be recognised from a tag only and one from pelage markings and a tag. However, observations in FN occurred on only a few days each breeding season, when the area was scanned for known animals. Thus, these females may have been present but not observed. None of the three branded females from FN that were not observed in their year of conception had paternities assigned to their pups.

For mothers in SA, where the greatest observational effort was directed, no known mother (out of 7) that was not seen in her year of conception had a pup that was assigned a father, whereas 50% of mothers (38/76) that were observed in the year of conception had pups assigned a father. This difference was not significant, possibly due to the low sample size ($\chi^2 = 2.04$, d.f. = 1, p = 0.101, with Yates' correction). A more conservative approach would be to use all mothers from SA that were not observed in the year of conception, thereby including females that may have been present but were unknown at that time (n = 62). When these females were included in the analysis, females that were observed in the year of conception were significantly more likely to have their pup assigned a father than those that were not ($\chi^2 = 6.18$, d.f. = 1, p = 0.007).

The relatedness of mothers and fathers assigned from parentage analysis (0.021 ± 0.169, n = 71) was not significantly different from random pairs (Figure 6.3; z = 0.68, p = 0.500). Surprisingly, the distribution of parental relatedness showed a small peak at r = 0.4 (Figure 6.3). This indicates relatedness greater than half siblings and consequently some degree of inbreeding. Approximately 20% (14/71) of parents were related according to the relaxed definition of relatedness (r > 0.155) while 7% (5/71) were related according to the strict definition (r > 0.314) (as described in Chapter 2).

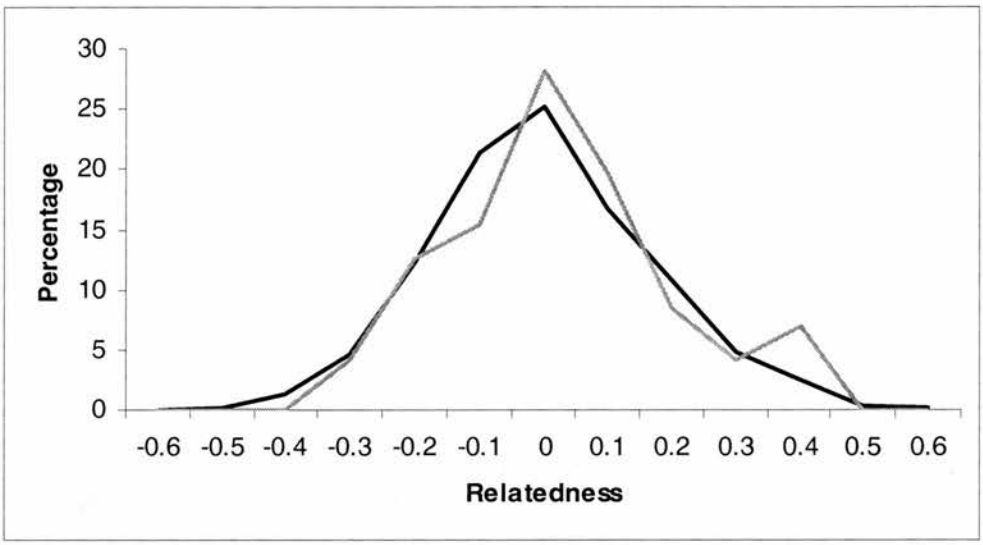

Figure 6.3: Distribution of relatedness values of random pairs (black line) and parents of sampled pups (grey line).

6.4.3 Observed sexual interactions

Sexual interactions were recorded from 1998 to 2003, with a total of 213 different pairs recorded, of which 115 were genotyped (Table 6.6). Within a year, the number of times each pair was recorded having a sexual interaction ranged from one to seven (Figure 6.4). In four cases, pairs were observed having a sexual interaction in more than one year. For three of these pairs, interactions took place in two consecutive years while the fourth took place two years apart. Within a given year, most females were only observed in sexual interactions with one male (n = 148), although some females were observed with two (n = 20), three (n = 8) or four (n = 2) different partners. Males were also observed having sexual interactions with more than one female in a given year (two n = 21; three n = 10; four n = 7; five or greater n = 12) but most were only observed with one female (n = 58).

Table 6.6: Number of males and females recorded as having a sexual interaction in each year and the number of pairs these individuals formed. Numbers in brackets are the number of individuals or pairs for which genotypes were available. The totals refer to the total number of different females, males and pairs that were observed over all years and are therefore not the sum of the columns, since the same individuals or pair may have been observed in more than one year.

Year	Number of individuals		Number of pairs	
	Females	Males	Attempted copulations	Copulations
1998	9 (9)	11 (1)	2 (0)	9 (1)
1999	7 (5)	6 (3)	1 (0)	6 (2)
2000	42 (22)	25 (15)	10 (2)	44 (16)
2001	36 (27)	22 (16)	6 (5)	34 (21)
2002	39 (31)	17 (14)	5 (1)	46 (36)
2003	36 (31)	19 (16)	8 (4)	37 (29)
Total	143 (94)	59 (30)	41 (12)	172 (103)

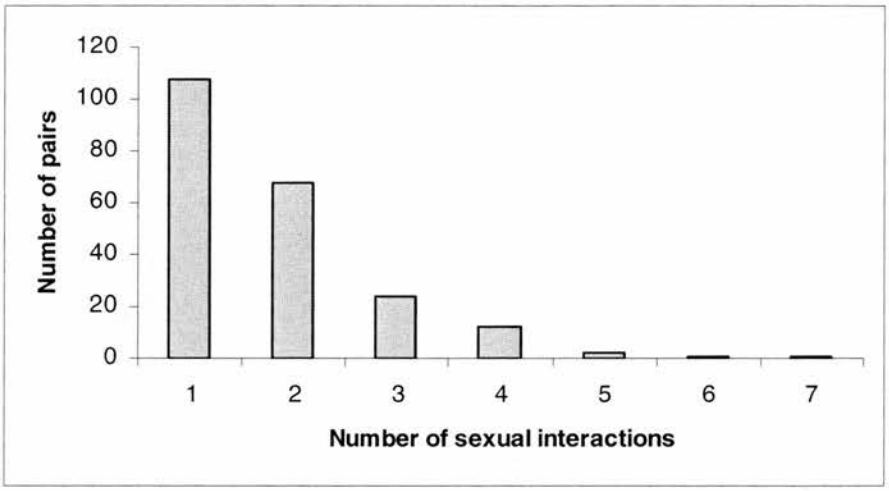

Figure 6.4: The number of recorded sexual interactions within a year (including attempted copulations and copulations) for each male-female pair.

Genotyped pairs that were observed having a sexual interaction (n = 115) were composed of 76 different females and 23 different males. The relatedness of pairs observed having a sexual interaction (0.000 ± 0.160) was not significantly different from random (Figure 6.5; $z = -0.46$, $p = 0.480$). This was also true when only pairs that were observed copulating were included (-0.002 ± 0.156; $z = -0.55$, $n = 103$, $p = 0.580$; Figure 6.5). Approximately 17% (20/115) of pairs having a sexual interaction were related according to the relaxed definition of relatedness ($r > 0.155$) while 4% (5/115) were related according to the strict definition ($r > 0.314$) (as described in Chapter 2).

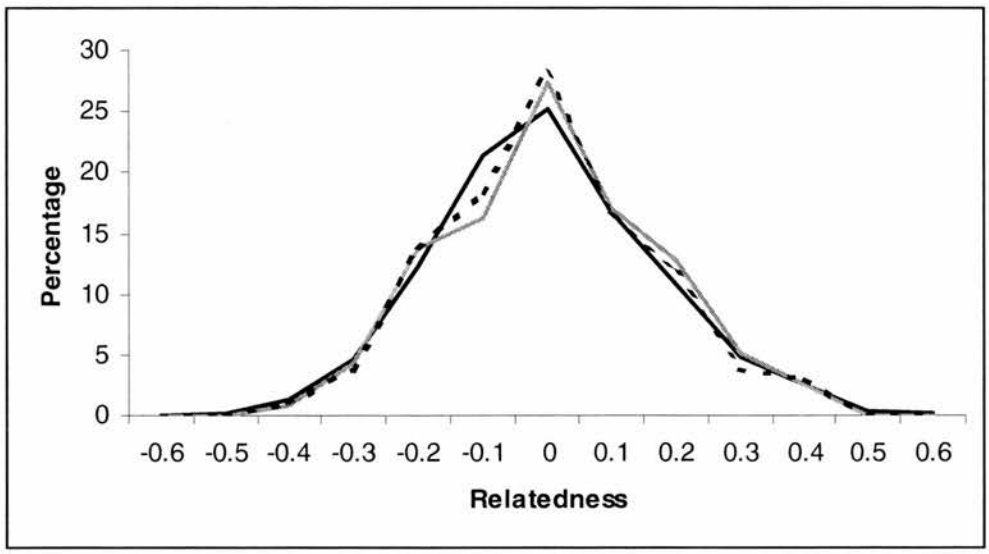

Figure 6.5: Distribution of relatedness values of random pairs (solid black line), male-female pairs observed having sexual interactions (solid grey line) and only those pairs observed copulating (dashed black line).

There were 20 pairs observed having a sexual interaction for which all individuals (females, males and pups in the following year) were genotyped. This included 15 different females and 13 different males. There were 17 observed copulations, of which 11 (65%) resulted in parentage. Ten of the 11 copulations resulting in parentage were recorded as successful while the other one was not. Of the copulations that did not result in parentage, four were recorded as successful and two as unsuccessful copulations. Over the season, three of the females were observed copulating with two different males. The first male to copulate was the father in one case, and in the other, the second male was the father. In the third case, neither male was the father. Three attempted copulations were also observed. In two instances, the male was the father of the pup born the following year to the females he was observed with. In the third, the female was seen copulating with another male that was the father. Overall, females were observed having sexual interactions with the male that fathered their next pup 13/15 (87%) times.

Pairs observed in sexual interactions that resulted in parentage were not less related than those that did not (Mann-Whitney U test: $Z = -0.56$, $N_1 = 13$, $N_2 = 7$, $p = 0.602$). Indeed, two copulations resulting in parentage occurred between pairs with relatedness higher than half siblings.

6.4.4 Male attendance in focal areas

A similar number of different males were observed over the season in the WP (n = 5) and the EP (n = 4) focal areas in 2002, while over twice as many were observed in the WP focal area in 2001 (n = 12). In addition, there were three days in 2001 in the WP where an unidentified male was observed and one day in the WP in 2002. Of the 12 identified males in WP 2001, all but two were genotyped. One of the males that was not genotyped was present for one day and the other was present for two days. One identified male, that was present for one day only, was not genotyped in WP 2002. All males were identified and genotyped in the EP 2002. Details of the number of focal females and the proportion genotyped can be found in Chapter 5.

While six different males were observed in one day in the WP in 2001, the maximum number observed in 2002 was three in the WP and two in the EP (Figure 6.6). There were also several days where no males were observed in the EP in 2002 and in the WP in 2001 (Figure 6.6). There was considerable variation in the length of stay of different males. One male (A3) was present on most days in the WP 2002 (observed on 19 out of 24 days) and was often the only male observed (14 days). Similarly, one male (B2) was observed in the EP 2002 on 12 out of 18 observation days and was the only male observed on 10 days. In the WP in 2001, the maximum number of days a male was observed for was seven (out of 16), which occurred in two instances. One of these males was the only male in the focal area on two days, while the other never was.

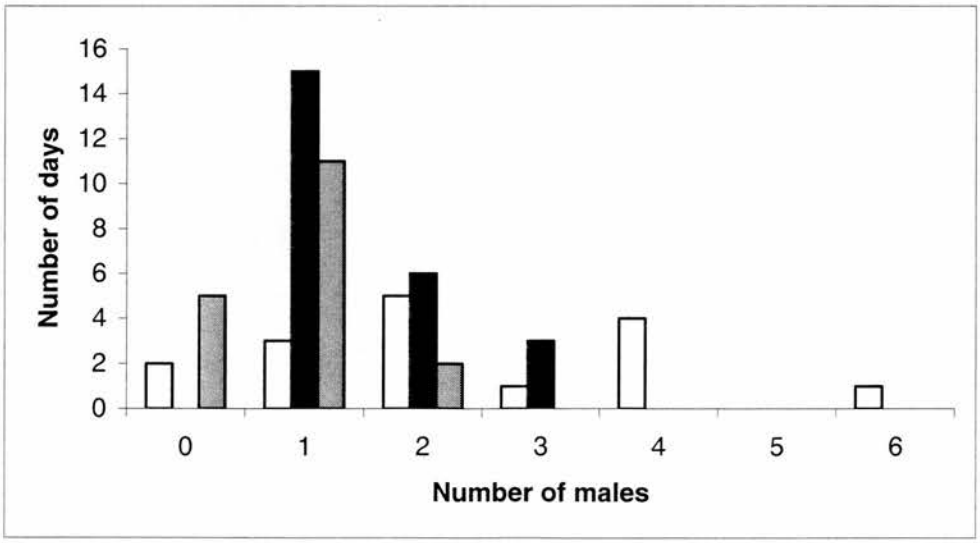

Figure 6.6: The number of males that were present on observation days in the WP 2001 (white bars), WP 2002 (black bars) and EP 2002 (grey bars).

Three males, known as Ziggy, Sneck and A3, were observed in the WP in 2001 and 2002. Ziggy was present for seven days in 2001 and in four days in 2002, Sneck was present for two days in 2001 and seven days in 2002 and A3 was observed on only one day in 2001 but for 19 days in 2002. Both Ziggy and Sneck were also observed in the EP in 2002, although only for one day each.

At the start of lactation, the relatedness of males to females observed in the focal areas (0.004 ± 0.157) was not significantly different from random ($z = 0.21$, $n = 82$, $p = 0.390$). Similarly, the relatedness of males to females during oestrus (-0.003 ± 0.121) was not significantly different from random ($z = -0.33$, $n = 84$, $p = 0.378$). At least one of the males that were present at the start of lactation was also present during oestrus for 22 of the 31 females that were observed over both periods.

6.4.5 Female-male aggression

A total of 449 aggressive interactions were recorded between males and females and all but 24 were initiated by females. The seasonal rate (per hour, per female) of aggressive interactions between males and females was similar in all years but was highest in the WP in 2002 (Table 6.7). However, a single male that was present for one day only in the WP in 2002 caused a very high level of disturbance that resulted in 74 aggressive interactions in one day, compared to a median number of interactions per day of 5.5. If this day was removed, the seasonal rate of interactions becomes 0.14, which is still higher than the other focal area and year.

Table 6.7: Aggressive interactions between males and females in the focal areas.

Area	Year	Aggressive interactions			
		Total	**During oestrus**	**Leading to sexual interactions**	**Seasonal rate**
WP	2001	129	18	9	0.11
WP	2002	255	90	10	0.17
EP	2002	65	6	0	0.09

The daily number of interactions was not significantly different between years in the WP ($F_{1,33} = 1.92$, $p = 0.176$) or between focal areas in 2002 ($F_{1,32} = 0.23$, $p = 0.635$). Between years in the WP, the number of males ($F_{1,33} = 10.17$, $p = 0.003$) and the number of females ($F_{1,33} = 5.58$, $p = 0.024$) observed each day explained a significant amount of variation, but not the number of hours males were observed ($F_{1,33} = 3.00$, $p =$

0.092). However, between focal areas in 2002, the number of hours that males were observed explained the greatest variation in daily interaction rates ($F_{1,32}$ = 8.83, p = 0.006). The number of females observed each day also explained a significant amount of variation ($F_{1,32}$ = 4.84, p = 0.034) but not the number of males ($F_{1,32}$ = 0.39, p = 0.535).

Of the aggressive interactions, 114 occurred when the female was known to be in oestrus and 19 led to either attempted copulations or copulations (Table 6.7). In the WP in 2001, half of all aggression during oestrus led to a sexual interaction, while in 2002 in the WP only 11% did. As discussed above, this may be partially explained by one male in the WP in 2002 that was present for one day and caused a large amount of disturbance later in the season when many females were in oestrus. There were 29 recorded aggressive interactions between that particular male and females that were in oestrus. However, even when these results were removed, only 10/61 (16%) aggressive interactions led to sexual interactions.

In 2001, the nine sexual interactions (six copulations and three attempted copulations) involved seven different females and four different males. Two females were estimated to be outside oestrus (days 11 and 18) when the sexual interactions occurred while lactation days could not be estimated for another two. In the WP in 2002, eight females and five males were observed in the 10 sexual interactions, of which four were copulations and six were attempted copulations. All females except one (day 12) were estimated to be in oestrus when sexual interactions occurred. Additional copulations were also recorded within the focal areas outwith the observation period in the WP (n = 4) and the EP (n = 3) in 2002.

The total number of aggressive interactions per female was greater during oestrus than earlier in lactation (t- test: t = -2.54, d.f. = 15, p = 0.023). Aggression was greatest immediately before (day 12) and during oestrus (day 13 to 17), although there was also an unexpectedly high rate of aggression on day nine (Figure 6.7). Aggression also tended to be higher near the beginning of lactation but was lower after oestrus on day 18 (Figure 6.7). One explanation for the higher rate of aggression during oestrus could be a greater number of males that were present at that time. For individual females, there were significantly more males present when they were in oestrus than at the start of

lactation (Wilcoxon signed ranks test: Z = -2.60, n = 31, p = 0.009), suggesting this may indeed be the cause of greater female aggression during oestrus.

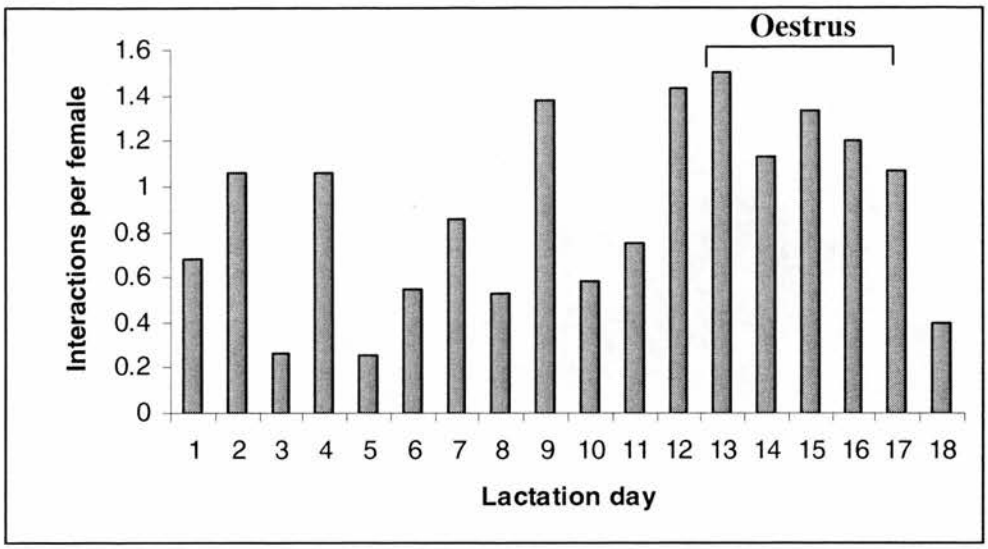

Figure 6.7: Number of inter-sexual aggressive interactions per focal female observed on each day of lactation, up to day 18, for all focal areas combined.

The relatedness of pairs that interacted aggressively during oestrus but did not have sexual interactions, was not significantly different from those that did (Mann-Whitney U test: Z = -0.57, N_1 = 12, N_2 = 14, p = 0.595). This was also true when attempted copulations were categorised separately from copulations (Kruskal-Wallis test: χ^2 = 0.46, d.f. = 2, p = 0.802). The males that females were observed mating with were not less related than the males (present during oestrus) that she was not observed mating with (Wilcoxon signed ranks test: Z = -0.62, n = 11, p = 0.575). (Three females were excluded from this analysis because the male they mated with was the only male present in the focal area over their period of oestrus.) Similarly, the females that males were observed mating with were not less related than the females (in oestrus) that they were not (Wilcoxon signed ranks test: Z = -0.77, n = 9, p = 0.500).

6.4.6 Patterns of parentage in focal areas

The most complete sampling of pups born in a focal area was in the WP in 2001 (Table 6.8A). Within focal areas, 13/20 (65%) sampled pups were assigned a father, which was higher than the overall percentage in SA (Table 6.5). One male fathered four pups that were born in the WP in 2001, while two of the pups born in the EP in 2002 were paternal half siblings (Table 6.8A). An additional pup in the EP was assigned the same father as the two other pups at the 80% confidence level, using 136 candidate males.

There were no mismatches between the male's and pup's genotypes, suggesting three out of the four sampled pups were paternal half siblings. One male fathered a pup in the WP in 2001 and in the EP in 2002. None of the mothers in the WP in 2001 and only two of the mothers in 2002 were observed with the father of their pup. In only one case was the mother observed with the father during oestrus, creating the potential for full siblings to be born in consecutive years.

Table 6.8: Paternities for A) pups born and B) pups conceived in the focal areas. Numbers in brackets indicate for A) the percentage of pups observed in the focal area that were sampled and for B) the percentage of known females observed in the focal area for more than one day with a pup sampled the following year.

Area	Year	Pups sampled	Fathers identified	Different fathers
A) Pups born				
WP	2001	11 (41%)	8	5
WP	2002	5 (17%)	2	2
EP	2002	4 (20%)	3	2
B) Pups conceived				
WP	2001	6 (25%)	4	3
WP	2002	2 (6%)	1	1
EP	2002	1 (4%)	0	0

The most complete sampling of pups conceived in a focal area was also the WP 2001 (Table 6.8B). Overall, 5/9 (56%) sampled pups conceived in a focal area were assigned fathers. This was also greater than the percentage of pups assigned a father for the entire SA (Table 6.5) but was similar to the percentage of pups with mothers observed in their year of conception in SA that were assigned a father (50%). Only one male fathered more than one pup, which is unsurprising given the low number of pups sampled. Most of the mothers of the nine sampled pups were not observed during oestrus (only one in each focal area or year), either because they left the focal area (n = 4) or oestrus occurred after the field season ended (n = 2). Two of the mothers that were not observed in the focal area during oestrus, but were known to be close by, had pups that were assigned fathers that were observed in the focal area. In one case, the mother was observed copulating with the father. When the mother was observed during oestrus, she was observed copulating with the father of her pup in the subsequent year in the WP in 2001 and 2002.

6.5 Discussion

This study combined behavioural observations of grey seal behaviour with paternity analysis. Previous studies have indicated that mate choice may play an important role in the grey seal mating system (Amos et al. 1995; 2001b) and that many pups are not fathered by sampled males (Amos et al. 1993; Worthington Wilmer et al. 1999; Lidgard et al. 2004). Here, no evidence was found to support the hypothesis that grey seals prefer to mate with less related, genetically dissimilar individuals at either the pre- or post-copulatory stage. However, several patterns of paternities were identified that could explain some of the shortfall in fathers previously reported in the North Rona breeding colony (Worthington Wilmer et al. 1999).

Within the colony, pairs that were observed mating were not less related to each other than random, suggesting the absence of pre-copulatory mate choice (Figure 6.5). Moreover, given the choice of individuals in the focal area, neither females nor males chose to mate with those they were least related to. There was also no indication that females chose to pup in locations defended by males that were less related to them than average. This was despite the finding that at least one of the males present when a female gave birth was usually present when she was in oestrus. However, analyses of behaviour in focal areas relied on small sample sizes. A colony-wide analysis of the relatedness of males to females within their defended area is necessary to draw any firm conclusions.

Another strategy females may use to select genetically dissimilar males is post-copulatory mate choice (Zeh & Zeh 1997; Tregenza & Wedell 2000). However, parents of pups were not less related to each other than average. Furthermore, observed sexual interactions that led to parentage were not between less related pairs than those interactions that did not result in parentage. Thus, females do not seem to be using post-copulatory mechanisms to choose the most genetically dissimilar mate. In fact, several instances of inbreeding were detected (Figure 6.3). Inbreeding may occur in the colony if one or both of the sexes are natal site faithful, because high adult site fidelity and longevity can result in an overlap of generations.

Individuals with higher heterozygosity are generally believed to have higher fitness (Hansson & Westberg 2002; Coltman & Slate 2003) and in both grey seals and harbour

seals *Phoca vitulina*, pups with higher genetic diversity have a greater chance of survival (Coltman et al. 1998; Bean et al. 2004). Therefore, there would appear to be a fitness advantage for females to mate with genetically dissimilar males. In grey seals, the mechanisms for assessing relatedness may not be highly developed and there are several lines of evidence suggesting they are unable to recognise kin (as discussed in Chapter 5). This could also indicate that selection for assessing relatedness, either behaviourally or cryptically, is low and inbreeding is not as detrimental in this species as previously considered (e.g. Amos et al. 2001a,b). Several other recent studies have also failed to detect mate choice for genetically dissimilar males, even when inbreeding depression occurs (Hosken et al. 2003; Feldheim et al. 2004; Jennions et al. 2004). More studies are needed to ascertain how commonly females choose males based on genetic compatibility in the wild, especially at the post-copulatory stage.

If females do not chose mates based on genetic dissimilarity what type of mate choice, if any, are female grey seals using? In terrestrially breeding pinnipeds, as with most polygynous mammals, females are usually believed to choose males with specific qualities, such as dominance or control of resources (Emlen & Oring 1977; Andersson 1994). For instance, female elephant seals *Mirounga spp.* may compete for access to larger harems that not only provide more protection but may also result in matings with higher quality males (Reiter et al. 1981; McMahon & Bradshaw 2004). It has also been suggested that females incite male competition in order to assess quality (Anderson et al. 1975; Cox & Le Boeuf 1977; Boness et al. 1982). Furthermore, genetic analysis has provided support for highly polygynous mating systems in some terrestrial pinnipeds, with dominant males gaining most paternities (Hoelzel et al. 1999; Hoffman et al. 2003; Fabiani et al. 2004). Similarly, grey seal pups are often fathered by dominant males indicating some females use this trait to select mates (Worthington Wilmer et al. 1999; 2000; Lidgard et al. 2004; this study). Nevertheless, not all pups are fathered by males expected from behavioural observations, suggesting female mate choice could exist for other traits (Amos et al. 1995; 2001b; Goldsworthy et al. 1999). In a mixed breeding colony of fur seals *Arctocephalus spp.*, female mate choice, at least for conspecific males, has previously been identified (Goldsworthy et al. 1999). A recent study by Hoffman et al. (2003) also raised the possibility that females may choose to mate with males outside their usual breeding colony, when not constrained by a pup, in order to increase genetic diversity. However, the possibility of mate choice for genetically

dissimilar mates has not been investigated in other pinniped species. Nevertheless, there are many other hypothesis as to why females may mate more than once and with males exhibiting alternative mating strategies, some of which are applicable to grey seals and other pinnipeds (reviewed in Reynolds 1996; Qvarnström & Forsgren 1998).

One possibility is that female grey seals do not exercise mate choice but are either forced to mate with males or find it less costly than resisting (Ambs et al. 1999). For instance, refusing males could result in injury or substantial energy costs. Although grey seals are not as size dimorphic as some pinnipeds, where males can easily overpower and cause harm to females (e.g. northern elephant seals *Mirounga angustirostris*, Le Boeuf & Mesnick 1990), some observations on Sable Island suggest that females may be forced to mate with males as they leave the colony (Boness & James 1979). Conversely, females have occasionally been noted soliciting matings from males (Anderson et al. 1975, S. Twiss pers. comm.).

Another hypothesis is that females mate with more than one male to ensure sufficient viable sperm is obtained. Specifically, dominant males may suffer from sperm depletion, making it beneficial for females to mate more than once (Preston et al. 2001; Wedell et al. 2002). Females often mate more than once with the same male suggesting that one copulation may not be sufficient for fertilisation (Figure 6.4; Boness & James 1979). Furthermore, a male may be sterile or have sperm that is genetically incompatible with the female (Jennions 1997). Poor quality sperm has been suggested as an explanation for the low reproductive success rate seen in some northern elephant seal alpha males, as this species has undergone an extreme population bottleneck (Hoelzel et a. 1999). Finally, sperm competition in females that mate with more than one male may increase the fitness of offspring if sperm competitiveness is genetically correlated with offspring fitness (Jennions & Petrie 2000; Hosken et al. 2003). Unfortunately, there are few data at present to either support or refute these hypotheses about multiple mating in grey seals.

Some evidence, though, suggests a more complex picture in which female grey seals are actively choosing mates. By modelling genetic data, Amos et al. (2001b) found evidence that females choose mates that are less genetically similar to previous partners (but not to themselves) than expected. However, this includes only the subset of females

that does not produce full siblings in multiple years. Thus, there is some evidence for a complex system of mate choice, potentially including behavioural pre-copulatory choice for the same father and post-copulatory choice for different fathers.

A greater number of full siblings than expected has previously been found on North Rona; this has been attributed to active mate choice by individuals (Amos et al. 1995; Worthington Wilmer et al. 2000). Only four sets of full siblings were identified in this dataset, making it difficult to infer any general patterns. (Other full siblings may have been present but did not have fathers that were sampled). Mothers of full siblings did not appear to be more site faithful than average (recently estimated at ~ 40 m; Pomeroy et al in press; Chapter 3). Indeed, between conception years, one female moved over 70 m. This tentatively supports the hypothesis that individuals are actively choosing the same mates in multiple years, as opposed to a passive mechanism based on site fidelity.

Males of polygynous species are unlikely to exhibit mate choice unless the costs of mating are high (Johnstone et al. 1996; Wedell et al. 2002; Werner & Lotem 2003). Male grey seals copulate infrequently, and copulations are relatively long (average duration is ~ 19 min on North Rona) suggesting males may be restricted in the number of females they can mate (Anderson & Harwood 1987). Therefore, dominant males may be unable to mate with all the females in their occupied area if several females come into oestrus at the same time. However, this study found no evidence for male mate choice. Males within the focal areas were not less related than average to focal females that were in oestrus, nor did they preferentially mate with less related focal females. Nevertheless, males may exhibit mate choice for other female characteristics.

Paternity analysis of grey seals from the North Rona colony has previously been conducted using a considerably larger sample size (290 males and 507 mother-pup pairs; Worthington Wilmer et al. 1999). Samples were also taken over more years than in the current study (9 vs. 4). Overall, results from the two studies, using samples taken from throughout the colony, appear extremely similar. Here, 39% of all sampled pups were assigned fathers (Table 6.2) while the previous study assigned fathers to 36% of pups (Worthington Wilmer et al. 1999). This is somewhat surprising given the larger number of males sampled in the Worthington Wilmer et al. (1999) study. Less than half of the dominant males observed in the Study Area and Fianuis South were sampled each

year in this study (Table 6.1), while in the study by Worthington Wilmer et al. (1999) up to 80% of all males were reported to have been sampled in this area of the colony. This suggests that incomplete sampling may account for some of the missing fathers but other sources are still necessary to explain the shortfall in paternities detected in both studies.

One reason why fathers were not sampled may be that pups, born to females that were absent in their year of conception, were not fathered by males from North Rona. No female that could confidently be said to be absent during the field season had a pup assigned a father. Moreover, for mothers in the Study Area, those that were observed in their year of conception were more likely to have the father of their pup sampled than those that were not. Since the Study Area is intensely studied, in some cases, females may be newly identified in the year they gave birth because they were not present in the previous year. Only 7% (6/82) of known females were not present on North Rona in the year they conceived, which is consistent with high site fidelity and birth rates in this species (Pomeroy et al. 1994; 1999). Thus, pups from these females will only account for a small proportion of missing paternities. However, the generality of these findings need to be confirmed by a much larger sample size, including greater numbers of males from North Rona that could have fathered these pups.

Other pups may not be assigned fathers due to spatial patterns of sampling. Few pups born in Fianuis North were assigned fathers, while many paternities were assigned to pups from Fianuis South (Table 6.5). Although detailed information on the location of individual males was not available, only one male was known to have been sampled in Fianuis North, which may explain the low proportion of paternities assigned in this region. Furthermore, paternal half siblings were always conceived in the same region. This suggests the region within the colony in which males and mother-pup pairs were sampled may be very influential in the determining patterns of paternities. Conversely, Worthington Wilmer et al. (1999) reported that the same proportion of pups from the north and south of the colony were assigned paternities, despite more males being sampled in the south.

Patterns of paternity also occurred on a finer spatial scale. Females with pups fathered by the same male were often observed in close proximity to each other in their year of

conception. This suggests these females were within an area occupied by the same male. However, females may also mate with neighbouring dominant males (Boness & James 1979; Boness et al. 1982; Amos et al. 1993). Paternal half siblings were also usually born within the same region of the colony and sometimes very close together, which is expected due to high site fidelity of females (Pomeroy et al. 1994; in press). Paternal half siblings were found close together within the focal areas as well. For instance, three out of the four pups in the East Pools had the same father.

A greater percentage of pups within the focal areas were assigned paternities (~ 60%) than within the Study Area as a whole (Table 6.5; 6.8). As focal areas were in central locations in the colony, this could suggest dominant males were more successful in these specific locations. Differential spatial reproductive success by dominant males has been identified on Sable Island, where females in certain locations (near the beach) were more likely to have pups fathered by their dominant male (Ambs et al. 1999). Therefore, one important area of future research is to identify whether females pupping in certain locations in the North Rona colony are more likely to have pups sired by dominant males. If this were the case then it may affect patterns of reproductive success derived from paternity analysis. For instance, if pups from certain locations were more easily sampled (e.g. the edges of the colony) the sample may not be representative of the colony. This could explain the difference in paternity assignment between the focal areas (65 – 56%) and the entire sample (39%).

The years that males and mother-pup pairs were sampled may also be important in assessing patterns of paternity. In 1999, when few genotyped males were observed, paternity assignment was also low (Table 6.1; Table 6.2). Moreover, males that were observed in the colony in the previous year were more likely to be assigned paternity than those that were not (Table 6.4). This suggests that a low sampling effort in one year will lead to fewer paternity assignments the next year, even though many males are observed in more than one season. In the study by Worthington Wilmer et al. (1999), most males were sampled in two breeding seasons, while mother-pup pairs were sampled in those two years and in earlier breeding seasons. Thus, for many pups, there was a low sampling effort of males the year they were conceived, which could result in misleading patterns of reproductive success. For example, Gemmel et al. (2001) reported that aquatic mating was likely to be an important mating strategy in the

Antarctic fur seal because many pups were not fathered by territorial males. However, samples were only taken over a two year period and thus males were not sampled in the year that many of the sampled pups were conceived. A longer-term study found that alternative mating strategies were unlikely to play a role in the breeding system of this species (Hoffman et al. 2003). Thus, the temporal aspect of sampling needs to be taken into account when drawing conclusions about reproductive success.

Males that were assigned at least one paternity in a season were observed in the colony for a greater length of time in the previous year than those that were not (Figure 6.2). This agrees with observations that males that stay ashore longest achieve the greatest number of matings (Godsell 1991; Twiss et al. 1994). Copulations that were observed in the colony also led to parentage in the majority of cases, as was found by Amos et al. (1993). Only three attempted copulations were observed, but two involved the father of the pup in the following year. Some of the copulations (and attempted copulations) did not result in parentage because a female was observed interacting with two different males. Combining results with the study by Amos et al. (1993), females were observed having a sexual interaction with the father in 19/24 (79%) of cases. However, only 19/28 (68%) of sexual interactions resulted in parentage.

As is typical of grey seal colonies, the rate of inter-sexual aggression was low (Boness et al. 1982; Anderson & Harwood 1985; Tinker et al. 1995; Redman 2002). Seasonal rates of aggression between males and females reported by Redman (2002) for the 1998 and 1999 breeding seasons on North Rona (0.16 and 0.10 respectively) were comparable to those found here (Table 6.7). Seasonal rates of aggression were similar for female-female and male-females interactions in 2001 while female-female seasonal interaction rates were higher in 2002 (Table 5.3; Table 6.7).

The number of inter-sexual aggressive interactions recorded daily did not differ between years or focal areas. This is in contrast to the number of daily female-female interactions, which was greater in 2002 than 2001 (Chapter 5). In this case, the increase in aggression in 2002 was explained by fewer pools of water leading to a higher density of females in the focal areas. It is surprising that greater female density did not increase female-male interaction rates as well, as males moving among females would have caused greater disturbance. Males moving within the focal area or trying to approach a

specific female would also have encountered more females in oestrus in 2002 than 2001, which may explain why most interactions with females during oestrus in 2002 did not lead to sexual interactions. Patterns of male attendance in the focal areas also differed between years (Figure 6.6). Many different males entered the West Pools in 2001 while there was a more stable situation in 2002, with only a handful of different males being present. Females are less aggressive towards dominant than subordinate males (Boness et al. 1982), and many of the males in 2001 may have had lower levels of dominance. Furthermore, females may get accustomed to a certain male, while a constant change over of males could produce more aggression. Therefore, differences in male behaviour in the West Pools between 2001 and 2002 may be due to variation in dominance relationships and male identities between years.

The rate of inter-sexual aggression was highest late in lactation, when females come into oestrus (Figure 6.7). This is contrary to other studies of grey seals that have found that female aggression towards males remains constant or decreases late in lactation (Boness et al. 1982; Redman 2002). Although differences in the sex ratio can alter the rate of harassment by males and therefore the rate of inter-sexual aggression (Boness et al. 1995; Galimberti et al. 2000) the study by Redman (2002) was conducted on North Rona, in a similar part of the colony over several years, suggesting this is not the reason for the difference in results. It is possible, instead, that different methodologies are responsible. Aggression also tended to be more frequent near the start of lactation (Figure 6.7), which probably functioned to protect young pups against males that could cause them harm (Maestripieri 1991). The trend for mothers to be more aggressive when pups are younger was also identified for intra-sexual aggression in Chapter 5 (Figure 5.5) and agrees with the findings of other studies (Boness et al. 1982; Redman 2002).

In conclusion, this study found no evidence for mate choice based on genetic dissimilarity. Instead, females may be forced to mate with males or may gain other fitness benefits from mating more than once and with males exhibiting alternative mating strategies. The results of paternity analysis using a small sample size were similar to those using a larger number of individuals, suggesting that sampling across the colony results in approximately 39% of pups being fathered by sampled males. However, about 60% of pups could be assigned paternities in focal areas suggesting the

success of parentage assignment may differ with location. Furthermore, 50% of pups in the Study Area were assigned fathers when mothers were observed in the colony in the year of their conception. The years in which males are sampled in relation to mothers and pups may also affect the number of paternities assigned. This study provides a basis for further work to be carried out using more detailed information on female, male and pup locations. The potential biases identified in paternity assignment based on the sampling regime or differential spatial patterns of reproductive success may be investigated fully using specialist spatial analysis software. Further data on male behaviour through the breeding season also needs to be incorporated into future analysis to examine differences in reproductive success among individual males.

Chapter 7: General discussion

Studies of the molecular ecology of social organisms have frequently described the distribution of genetic variation within and between social groups (Ross 2001). This can provide information on the likelihood of both kin-biased behaviours and inbreeding being observed in the population (e.g. Kerth et al. 2000; Spong et al. 2002; Van Horn et al. 2004). Furthermore, many studies incorporating genetic data have been successful in explaining the evolution and maintenance of social behaviours, such as cooperation, as well as revealing information about breeding systems (reviewed in Reynolds 1996; Hughes 1998; Ross 2001). In this thesis, I examined the relationship between the behaviour of breeding grey seals and two genetic variables. Specifically, I investigated the spatial and temporal patterns of relatedness and genetic diversity of breeding females (Chapters 3 and 4). I also examined the effect of these genetic variables on aggressive behaviour between females (Chapter 5). Additionally, patterns of parentage and mate choice were described (Chapter 6). However, unlike much research in this area, in the grey seal, females do not exhibit complex social behaviour in the breeding colony. Nevertheless, a recent study found evidence of active associations between females between years (Pomeroy et al. in press) and passive associations are expected through high levels of site and temporal fidelity (Chapter 3; Pomeroy et al. 1994; 1999; in press; Twiss et al. 1994). Thus, grey seals on North Rona provide the opportunity to examine the effects of relatedness and genetic diversity on the behaviour of individuals in a population where a basic form of sociality may be present.

7.1 Relatedness and genetic diversity estimates

Relatedness and genetic diversity, two interrelated genetic measures, were used in this study. These measures are estimates of the degree of relatedness between individual seals and the relative inbreeding of individuals respectively. These estimates could be obtained more accurately using pedigrees (Pemberton 2004). However, like many wild study populations of long-lived species, pedigrees are not available for grey seals in the North Rona colony. Thus, the best obtainable relatedness and inbreeding estimates are those based on genetic data.

In Chapter 2, the ability of the 11 microsatellite loci used in this study to estimate relatedness between individual grey seals was examined. High variance is always associated with relatedness estimates (Lynch and Ritland 1999; Blouin 2003) and with

11 loci it was only feasible to differentiate with confidence between related and unrelated pairs. However, the level of accuracy achieved would have been sufficient to detect any biologically relevant differences in the relatedness of groups involved in comparisons throughout this study. Many studies using eleven or fewer microsatellite loci to estimate relatedness (with a similar average number of alleles and heterozygosity) have found differences in behaviour based on kinship (e.g. fostering behaviour in Antarctic fur seals *Arctocephalus gazella*, Gemmel 2003; aggression reduction in tammar wallabies *Macropus eugenii*, Blumstein et al. 2002; cooperative behaviour in carrion crows *Corvus corone corone*, Baglione et al. 2003). Nevertheless, if variation in behaviour was affected by small differences in relatedness, the variance in these microsatellite-derived estimates may be too great to detect a biologically important relationship.

While microsatellite-based relatedness estimators have been used for over a decade and have been rigorously examined (Queller & Goodnight 1989; Blouin et al. 1996; Lynch & Ritland 1999; Van de Casteele et al. 2001; Wang 2002), the development of microsatellite-based measures of inbreeding is relatively recent (Coulson et al. 1998; Coltman et al. 1999; Amos et al 2001a). When I began this thesis in 2001, several papers had recently been published correlating microsatellite heterozygosity, or genetic diversity, with traits that affect fitness; positive correlations were interpreted as evidence of inbreeding depression or heterosis (Coltman et al. 1998; 1999; Coulson et al. 1998; Slate et al. 2000; Amos et al. 2001a; Rossiter et al. 2001). However, in the past year, the ability of microsatellite heterozygosity to estimate inbreeding coefficients has been questioned (reviewed in Pemberton 2004). In several populations, individual microsatellite heterozygosity has been found to correlate only weakly, or not at all, to the inbreeding coefficient (Balloux et al. 2004; Slate et al. 2004; Markert et al. 2004). Indeed, unless the population exhibits high variance in the level of inbreeding and a large number of microsatellite loci are used (~ 200), significant positive correlations between inbreeding and heterozygosity are now thought to be unlikely (Balloux et al. 2004; Slate et al. 2004). This implies that often, microsatellite-based measures of genetic diversity do not accurately reflect genome-wide heterozygosity. Instead, correlations with fitness traits may arise though linkage with fitness-influencing loci that exhibit overdominance (Bierne et al. 1998; David 1998; Hansson & Westberg 2002). Therefore, when interpreting results using genetic diversity, it is necessary to be

aware that it does not necessarily reflect the level of inbreeding, but may indicate linkage to unknown genes.

Consequently, one area that future studies need to address is improving the quality of relatedness and inbreeding estimates. This may be accomplished by adding more markers to the dataset. However, several tens of microsatellite loci would have to be added to see a marked increase in the accuracy of relatedness and inbreeding estimates (Blouin et al. 1996; Slate & Pemberton 2002; Balloux et al. 2004). Further studies using microsatellite-based genetic diversity estimates also need to investigate the effect of individual loci and the likelihood of linkage to other loci that are under selection.

A better strategy to increase the accuracy of relatedness and inbreeding estimates may be the inclusion of pedigree data (e.g. Clutton-Brock et al. 2000; Marshall et al. 2002; Markert et al. 2004). A few mother-offspring pairs are known on North Rona through tagging studies (Pomeroy et al. 2000a). Comparing genotypes of pups sampled more than three years ago to adults sampled now could provide more pedigree information, as could paternity analysis. Several genetic programmes can also use genotypes to estimate the likelihood of pairs belonging to a relationship category (reviewed in Blouin 2003). However, constructing even a partially complete pedigree of the adults observed in the Study Area on North Rona would require a long-term programme that used an efficient method of recognising individual pups when they return as adults.

7.2 Effect of relatedness on behaviour

In Chapter 3, the distribution of genetic variation and patterns of relatedness of breeding females were investigated within the colony. There was little evidence of either fine-scale spatial or temporal genetic structuring, with the exception of the Fianuis South region (Figure 3.5; 3.6; Table 3.4). This was contrary to what I predicted, given the evidence for philopatry, natal site fidelity, and strong breeding site and temporal fidelity of females (Table 3.3; Figure 3.4; Pomeroy et al. 1994; 1999; 2000a; in press; Allen et al. 1995). Fine-scale kin clustering of many species has been attributed to strong philopatry to the natal breeding site (e.g. Surridge et al. 1999; Ratnayeke et al. 2002; Coltman et al. 2003) including some species that form temporary breeding assemblages (Kerth et al. 2000; Schjørring 2001). However, it appears that the precision with which

female grey seals return to their natal site is not sufficiently high to produce detectable kin clustering with the measures used.

Additionally, individuals may seek out kin if they gain fitness benefits from interacting with them (Baglione et al. 2003). However, there was no evidence that grey seal mothers preferentially pupped near relatives in the colony. Furthermore, behavioural data indicates that mothers did not alter their aggressive behaviour towards transient females based on relatedness (Figure 5.7). This suggests that mothers did not encourage relatives to pup close to them by reducing aggression.

There is other research, however, that suggests a general absence of genetic structuring within the colony was not surprising. Pomeroy et al. 2001 previously failed to find a correlation between the genetic relatedness of females and the distance between their pupping sites. In addition, several papers have reported a lack of genetic divergence between grey seal colonies that are in relatively close proximity to each other (e.g. east coast of Canada, Boskovic et al. 1996; Orkney Islands, Gaggiotti et al. 2002; Baltic Sea, Graves et al. submitted). This is probably due to both historic and current gene flow, as many colonies have fluctuated in size or been recently founded (Gaggiotti et al. 2002; Graves et al. submitted), and seals have been observed moving between breeding sites (Pomeroy et al. 2000a). These are also likely to be the reasons why no genetic differentiation between regions was detected on North Rona.

However, there was evidence for kin clustering in the Fianuis South region of the colony, which suggests strong natal site fidelity (Table 3.4; Figure 3.6). The kin clustering observed in this region of the colony may have been due to differences in habitat and lower female density. It is also interesting that a high proportion of pups were assigned paternities in Fianuis South (Table 6.4). Half of the pups (6 out of 12) were assigned the same father, making them paternal half siblings. It is possible that these two findings are connected. Higher levels of polygyny, resulting in a greater number of paternal half siblings, will increase the overall relatedness of pups. If females are philopatric to the natal region, this will also increase the relatedness of mothers, making kin clustering more likely. However, sample sizes for Fianuis South were small compared to the Study Area, especially for the paternity data. Future studies should

focus on this region to determine whether kin clustering does occur and whether this could be due to differences in breeding behaviour.

It was important to discover the extent of kin clustering before exploring whether aggression reduction occurs between females, because spatial patterns will affect the likelihood of this behaviour. Although data from one year suggested that aggression reduction between kin might occur, this was not supported by other analyses (Table 5.7; Figure 5.7; 5.11). Instead, higher rates of aggression were generally observed when females were near pools, showed greater movement within focal areas and were at higher densities (Table 5.7). Thus, as expected from the lack of kin clustering, there was little evidence that relatives were less aggressive towards each other (Figure 5.7; Table 5.7). The lack of kin-biased behaviour may also be due to poor kin recognition abilities. In addition, aggression usually occurred at low intensity and was typically infrequent (Table 5.3; 5.4; Figure 5.4). This resulted in a small amount of data for each female, with many female pairs not interacting at all. Moreover, the function of most aggression was not obviously apparent; nor were the costs and benefits associated with this behaviour. Additional research into the fitness effects of aggression in female grey seals may help to explain further the results from this study.

Individuals may assess kinship in order to increase their inclusive fitness (Holmes 1988; Waldman 1988) or to choose their optimal mate (Penn & Potts 1999; Tregenza & Wedell 2000; Mays & Hill 2004). This second possibility was investigated in Chapter 6. There was no evidence that either males or females preferentially mated, or associated, with members of the opposite sex with regard to relatedness (Figure 6.3; 6.5). Furthermore, several instances of inbreeding were detected (Figure 6.3). The proportion of inbreeding, estimated at between 7% and 20%, falls with the range reported for other mammalian populations, measured using pedigrees (Marshall et al. 2002). This suggests that grey seals may use other cues to assess the quality of mates.

Additionally, spatial and temporal patterns of the average relatedness of females to the colony were investigated in Chapter 4. This differed from the analyses in other chapters because it did not examine differences in behaviour between pairs based on their relatedness to each other. Instead, for each female, the average relatedness to all other sampled females in the colony was compared. There was no indication that females

with higher average relatedness to the colony consistently pupped together in the same locations or at the same time during the breeding season (Figure 4.2B; 4.4; Table 4.3; 4.5). Nor did they pup in sites with preferred topographic characteristics (Table 4.7B). Specifically, those females breeding in central areas of the colony did not have more relatives in the colony than those pupping in other locations, as had been found in a previous study (Pomeroy et al. 2001). This could indicate that females in these sites do not have greater reproductive success or breeding and natal site fidelity are not strong enough to maintain genetic structuring.

In summary, the effect of relatedness on the behaviour of individual grey seals was examined for three different behaviours: kin clustering, aggression reduction and mate choice. Overall, there was no strong evidence that behaviour was influenced by the relatedness of participants. This may be because kin-biased behaviours do not increase fitness or due to an inability of grey seals to accurately assess relatedness, or both. In addition, mothers with higher average relatedness to other mothers in the colony were not clustered in preferred habitat. Therefore, along with the general lack of kin clustering found in Chapter 3, results indicate there is little structuring of relatedness of mothers in the North Rona colony, with the exception of Fianuis South.

7.3 Effect of genetic diversity on behaviour

The spatial and temporal patterns of genetic diversity of breeding females were examined in Chapter 4; females with higher genetic diversity were predicted to cluster together in preferred habitats. There was some indication that mothers with similar genetic diversity clustered together in the same locations each year, although patterns were not entirely consistent (Figure 4.3; Table 4.3). I also found that, in two of the four years studied (2000 and 2002), mothers with higher genetic diversity pupped in locations with greater female density (Table 4.7B). In the remaining two years (1997 and 2001) there was a positive relationship between female density and the local spatial autocorrelation measure for genetic diversity (Table 4.7A). That is, locations where mothers within a 30 m radius had higher than average genetic diversity were also locations of greater female density (although this was only significant in one year). Greater density of females may reflect higher quality habitat, which suggests that those mothers with higher genetic diversity were able to gain access to preferred locations. As

results were not consistent across the four years examined, data from additional years would help to determine the consistency and biological relevance of this pattern.

Several other studies have found a positive relationship between genetic diversity, or inbreeding, and competitive ability (Meagher et al. 2000; Höglund et al. 2002; Tiira et al. 2003; Hoffman et al. 2004). However, the link between the competitive behaviour and fitness was more obvious in these studies than for pupping site characteristics of female grey seals. For instance, inbreeding decreases competitive ability in male house mice *Mus domesticus* and males with lower competitive ability fail to gain territories and obtain matings (Meagher et al. 2000). In grey seals, there is little direct evidence that pupping site characteristics affect the relative reproductive success of mothers with a year or area of a colony. Previous studies have shown that females prefer to pup in sites close to pools of water and access to the sea (Pomeroy et al. 2000b; Twiss et al. 2001). Mothers may also spend less time with pups when water is scarce within a region or time within the breeding season (Twiss et al. 2000a; Redman et al. 2001). One study also found that females an optimal distance from the sea had pups with higher growth rates (Pomeroy et al. 2001). Studies examining directly how the reproductive success of mothers in the North Rona colony is affected by pupping site characteristics, including female density, are needed to determine whether mothers pupping in these locations gain measurable benefits. Examining the characteristics of mothers (e.g. age, size) pupping in different quality sites at a fine-scale would also be useful. The inclusion of genetic diversity estimates in these analyses would further help to understand how these factors combine to influence reproductive success.

In Chapter 5, the effect of genetic diversity on aggressive behaviour was also examined. In one of the two years studied (2002), females with higher heterozygosity tended to be more aggressive (Table 5.7). Higher levels of maternal aggression could increase pup survival because it functions to move other females away from the pup (Christenson & Le Boeuf 1978; Ribic 1988). Thus, this may reflect a greater ability to defend the space immediately around themselves, and their pup, from other females. In addition, the positive relationship between aggression and genetic diversity may indicate a greater ability of females with high genetic diversity to access and maintain a position in high quality habitat. In general, the effects of inbreeding are more pronounced under stressful conditions (Audo & Diehl 1995; Crnokrak & Roff 1999). This may explain why there

was no relationship between aggression and heterozygosity in the other year (2001), as environmental conditions were less stressful.

7.4 Scale effects

Many of the analyses in this thesis used distance measurements and therefore often included a specific scale. When animals are divided into discrete groups, these may form the unit of study for fine-scale genetic analyses (e.g. bird leks, Petrie et al. 1999; Shorey et al. 2000; bottlenose dolphin *Tursiops spp.* alliances, Krützen et al. 2003; hyena *Crocuta crocuta* clans, Van Horn et al. 2004). Furthermore, in territorial species, only those individuals with neighbouring territories or home ranges are likely to interact (e.g. red grouse *Lagopus lagopus scoticus*, Watson et al. 1994; bank voles *Clethrionomys glareolus*, Mappes et al. 1995). However, grey seals mothers do not generally form discrete groups in the breeding colony and may change position during the lactation period. Therefore, a biologically relevant scale had to be chosen for analyses based on movement patterns of mothers.

In Chapter 3, the 10 m^2 grid cell in which a female pupped was used as her location, making this the finest scale possible for analyses. As pupping location was used, the distance at which females were likely to interact had to encompass movements over the entire breeding period. Therefore, to represent mothers that were likely to interact, mothers within a grid cell and the eight surrounding grid cells were chosen; this is equivalent to a 14 m radius. The choice of grid cells relied on a previous study indicating females usually remain within 10 m of their pupping site (Pomeroy et al. in press). In Chapter 5, females' locations were measured hourly (and to the nearest 2 m), and therefore the distance at which females were likely to interact only included movements made within an hour. From the data, I estimated that 95% of interactions took place between females within a 10 m radius (Figure 5.2). Therefore, in Chapter 5, females that were considered likely to interact were chosen as those within a 10 m radius of each other.

In some instances, employing a range of spatial scales may be the best way to identify fine-scale spatial patterns of genetic measures (e.g. Surridge et al. 1999; Coltman et al. 2003). Here, a range of spatial scales was used for spatial autocorrelation analyses (Chapter 4). The smallest scale chosen was 10 m, which represented mothers that were

likely to interact. However, the 10 m scale proved too small to detect meaningful patterns using the local spatial autocorrelation analysis because many individuals had few or no neighbours. Clustering of females with similar genetic diversity or average relatedness to the colony could be due to either individual competitive ability or passive processes, which may be detected at a larger scale. Hence, local spatial autocorrelation analyses were also conducted at a scale of 30 m. A larger scale was tested as well (50 m) but was too large to create meaningful results due to the high degree of overlap between the mothers that were included in comparisons for each location.

7.5 Paternity analysis

Genotypes were also used to assign paternity to sampled pups (Chapter 6). I examined the paternity data with respect to behaviour and sampling distributions. As with previous studies of grey seals (Amos et al. 1993; Worthington Wilmer et al. 1999; Ambs et al. 1999; Lidgard et al. 2004) and several other pinniped species (Hoelzel et al. 1999; Hoffman et al. 2003; Fabiani et al. 2004) the level of polygyny suggested by the genetic paternity analysis generally agreed with behavioural observations. Indeed, though sample sizes were small, most observed sexual interactions led to paternity, which agreed with a previous study (Amos et al. 1993). Patterns of both temporal and spatial sampling were found to have an impact on the proportion of paternities assigned. In the focal areas, which were in central locations in the colony, around 60% of pups could be assigned fathers whereas overall, parentage could be assigned to less than 40% of sampled pups (Table 6.2; 6.8). Males that were observed in the previous year were also more likely to be assigned a paternity than those that were not (Table 6.4). Similarly, females that were not observed in the previous year were less likely to have the father of their pup sampled. This suggests that the sampling regime, and identities of the individuals sampled, will affect the proportion of pups that can be assigned paternities, as has been found for other pinniped colonies (Ambs et al. 1999; Hoffman et al. 2003).

Future studies examining patterns of parentage will benefit from incorporating fine-scale information about male and female locations from the GIS of the North Rona colony as well as detailed behavioural data for individual males. Specifically, the factors that affect variation in reproductive success of males that hold positions within the colony could to be investigated further. For instance, do males that maintain a

position in the centre of the colony gain greater reproductive success than those in the periphery of the colony? Additionally, the time during the breeding season that males are ashore may affect their likelihood of fathering pups. The effect of sampling distribution could also be studied in greater detail by examining the distribution of sampled pups with respect to the location of sampled males during the preceding breeding season. This may provide an explanation for why some males were not assigned parentage or why some pups were not assigned fathers.

7.6 Behavioural findings

Although this thesis focussed on using genetic variables to understand behaviour, some interesting results emerged from the detailed behavioural observations. First, the rate of aggression observed for each female, towards other females, tended to be higher when her pup was younger (Figure 5.5). Aggression by females towards males was also higher when their pups were very young, but was greatest later in the lactation period when females came into oestrus (Figure 6.7). While the result for intra-sexual aggression agreed with other research, previous findings suggested that inter-sexual aggression was also highest at the start of lactation, not during oestrus (Boness et al. 1982; Redman 2002).

Seasonal female-female aggression rates were higher than female-male aggression rates in 2002, but were similar in 2001 (Table 5.3; Table 6.7). This is a result of a higher rate of female-female interactions in 2002, compared to 2001 (Table 5.3; Figure 5.4). The intensity of observed female-female aggressive interactions was also greater in 2002 (Table 5.4). The observed increase in aggression in this year was probably due to greater crowding around fewer pools of water (Table 5.8). This provides additional evidence that access to water is an important resource to females on the breeding colony (Twiss et al. 2000a; 2002; Redman et al. 2001). It also emphasises the importance of variation in environmental conditions on social interactions.

The results of the analyses examining factors affecting individual rates of aggression were very inconsistent between years (Table 5.7). As the two years that were studied differed in environmental conditions, the addition of several more years' data may indicate which factors are consistently important in affecting the rate of aggression. Furthermore, only two areas in the colony were examined, both of which centred on

relatively large pools of water. Examination of several different areas may provide a more complete picture of the colony. In particular, future studies could use focal areas in Fianuis South, where kin clustering was identified.

7.7 Conclusion

This study examined the possibility that kinship affects behaviour in a population without a highly developed social system. However, I found little evidence that the behaviour of female grey seals was affected by their relatedness to each other. Therefore, kin selection appears unlikely to be playing a major role in the development of sociality of females grey seals in the North Rona breeding colony. Even in the absence of complex social behaviour, distinguishing relatives can be important for selecting mates. However, there was no evidence that mate choice for genetically dissimilar partners occurred in the colony. Taken together, results using relatedness estimates provide no support for the hypothesis that kin recognition occurs between adult grey seals. There was some evidence that mothers with greater genetic diversity clustered in locations where female density was higher. Females with greater genetic diversity also tended to be more aggressive in one of the two years studied. This suggests that genetic diversity may correlate to some extent with competitive and maternal ability but further studies are needed to confirm this interpretation. Finally, including information on behaviour and sampling distribution in analyses of parentage assignment uncovered several explanations for the previously reported shortfall in paternities.

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