Molecular ecology of southern elephant seals (mirounga leonina): mating system and population genetics

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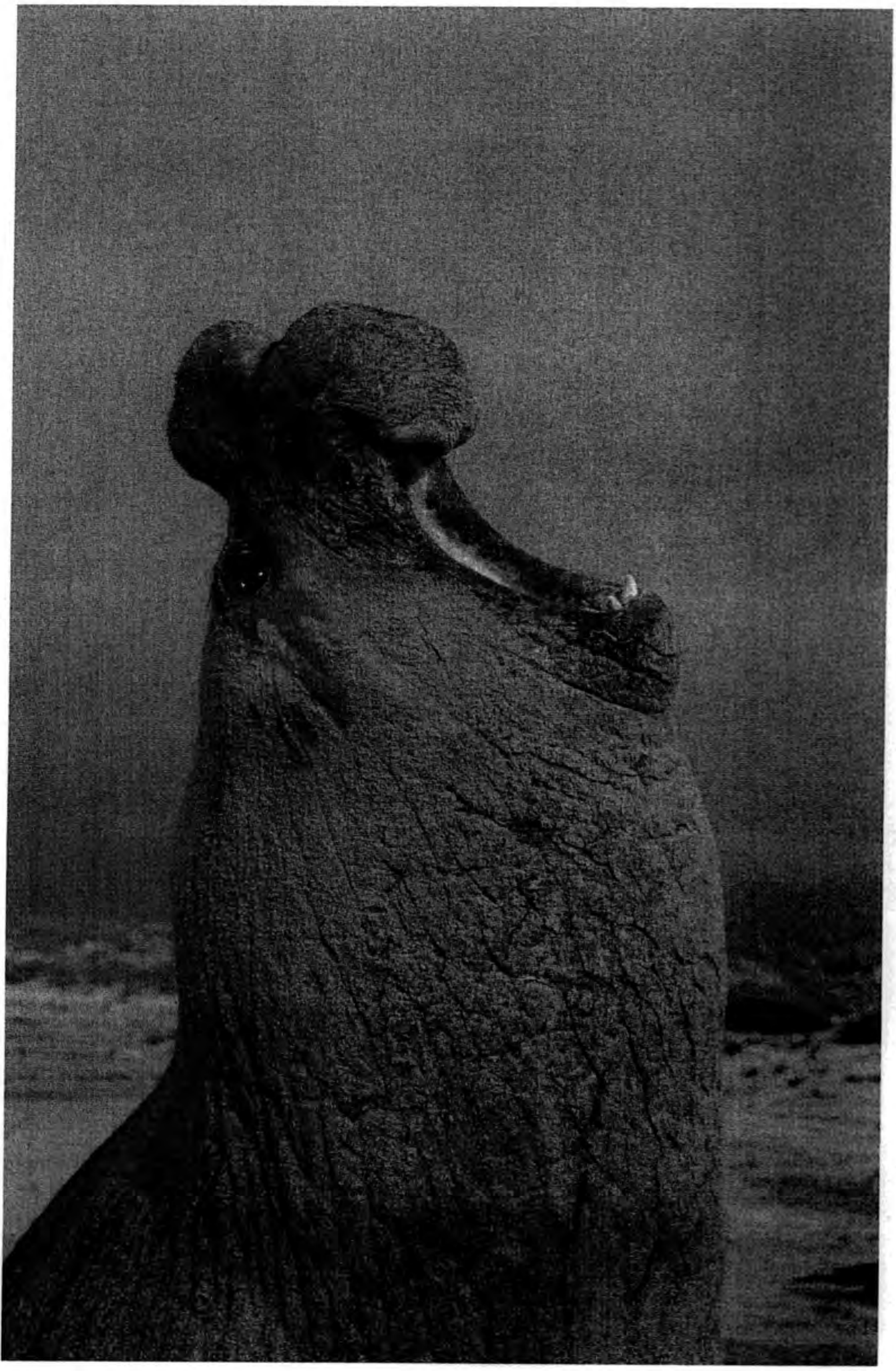

Molecular ecology of southern elephant seals (*Mirounga leonina*): mating system and population genetics

by

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Thesis abstract

The general aim of this research was the analysis of the mating system and the genetic structure of the southern elephant seal (*Mirounga leonina*). The specific objectives were to: 1) estimate the distribution of paternity and the relationship between behavioural and genetic indices of male reproductive success; 2) quantify the level of kinship among seals and investigate the presence of any genetic pattern that might be a consequence of the social structure of the population; 3) assess the genetic variation among and within the stocks of the Southern Ocean and analyse the pattern of individual dispersal.

The research was conducted at Sea Lion Island (SLI, Falklands), from 1996 to 1998. All breeding males of the colony, and females and pups belonging to seven harems ($n = 455$) were tissue sampled. The samples were analysed at nine microsatellite loci and likelihood based paternity analyses were conducted. Samples ($n = 46$) from Elephant Island (EI, South Shetlands) were also characterised at the same microsatellite loci, and the mtDNA control region (299bp) was sequenced in 57 seals from SLI and 30 from EI.

The success of the paternity inference was very high, as a father was found for 95.3% of the pups. Out of 183 assigned paternities, 151 (82.5%) were secure at the 95% confidence level and 32 (17.5%) at the 80% level. The distribution of paternities indicated an extremely polygynous system, with the majority of males achieving zero paternities and the harem holders siring up to 96% of the offspring in each harem (mean 78). Paternity was highly correlated with behavioural indices of mating success (R^2 0.80-0.99), and predicted individual paternities 60%-100% of the time in each harem.

Elevated relatedness values (R) within colony suggested some level of philopatry, though the low F_{st} indicated female dispersal between SLI and EI. Females from SLI showed a general higher level of relatedness among each other than did males. However, the harem structuring did not lead to any detectable genetic substructure within the population.

Genetic differentiation was found both within and among putative colonies of the Southern Ocean. The differentiation at mitochondrial markers was higher than at nuclear markers, pointing to a difference in the pattern of breeding dispersal between sexes. Despite the extensive mitochondrial variation ($\phi_{ST} = 0.5$), genetic evidence of male mediated gene flow was also found between SLI and Macquarie Island (MQ), as an adult male on SLI showed the same haplotype as a seal belonging to the very well defined MQ lineage. Given the pattern of genetic diversity and patterns of fidelity and dispersal in elephant seals, the most parsimonious interpretation is that the male travelled from MQ to SLI. He successfully reproduced in 1996 on SLI, fathering at least 18 offspring. His exceptional migration demonstrates that even populations separated by wide genetic and geographic distances can remain linked, and it is an example of the potential homogenising effect that dispersal and mating system can have on the genetic patterns of a population.

DECLARATION

The material contained in this thesis has not previously been submitted for a degree at the University of Durham or any other university. The research reported within this thesis has been conducted by the author unless otherwise indicated.

Acknowledgments

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This research is part of a very long project on elephant seals, and I owe a huge debt to my parents, who supported me at the beginning of this sometimes 'extreme' experience and helped me all the way through these years, regardless of the quality of my decisions. This thesis is dedicated to them.

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I. INTRODUCTION

In recent years, molecular genetic methods have become increasingly sophisticated and easily applied to studies at the species, population and subpopulation levels (Avise 1994, Sugg *et al.* 1996). Their application to the investigation of mating systems, reproductive success and relatedness between individuals in a population has provided new important information about the biology of many species, including genetic structure (Paetkau *et al.* 1999; Girman *et al.* 2001), dispersal (Lyrholm *et al.* 1999; Pardini *et al.* 2001), social structure and mating systems (Pemberton *et al.* 1992; Lebas 2001).

Several factors are responsible for the differentiation of gene frequencies among and within populations of a species. These factors involve gene flow, random genetic drift, various modes of natural and sexual selection, and the opportunity for recombination mediated by the mating system of the species (Avise 1994). In the traditional approach of population genetics, individuals mate randomly and biodiversity is assessed using populations as discrete units. However, in many species, random mating does not occur, dispersal and philopatry are sex biased and social organisation restricts the access to potential mates to only a few individuals (Sugg *et al.* 1996). Therefore, it is clear that genetic considerations require the study of the biological parameters and the natural history traits relevant to each population.

I.1 Genetics and reproduction

‘Reproduction, in a word, is the central problem in the life sciences, for it is the issue around which all other aspects of biology hinge” (Dunbar 1984).

Studies of the variance in reproductive success are critical to understanding demography, genetic structure and processes of selection in natural populations (Coltman *et al.* 1998). Sexual selection is the differences in reproduction that arise from variation among individuals in traits that affect success in competition over mates and fertilisations (Andersson 1994). High variance in reproductive success between and within sexes can be an indicator of sexual selection and be a major factor for the evolution of life history traits, morphology and mating system characteristics (Emlen and Oring 1977; Andersson 1994). Reproductive variance within sex indicates a potential for selection among males and females, and different selective pressures between sexes allow the evolution of phenotypic traits in the sex where selection is stronger. In polygynous species, variance in male reproductive success is large and may affect the rate of loss of genetic variation and alter the genotypic proportions from those expected with panmixia (Chesser 1991). In fact, the degree of polygyny can strongly influence the effective population size and hence the rate at which genetic drift may operate (because the rate at which genetic variation is lost is inversely proportional to the effective population size N_e).

Mating systems describe the ways in which individuals of both sexes interact to maximise their reproductive success (Davies 1991), and are best viewed as the outcome of the reproductive strategies of individuals rather than population or species characteristics (Clutton Brock 1989). Provided that only female parental care is required, males should maximise fitness by competing for as many mates as possible whereas females should maximise fitness by ensuring offspring survival and choosing 'good mates' (Boness *et al.* 1993). The extent of variation in mating and competitive behaviour, within and between populations, will depend on the adjustment of individual behaviour to differences in the social and ecological environment, and to variation in individual capabilities (Emlen and Oring 1977).

With the application of molecular techniques, much progress has been made in the study of mating systems at the level of the individual (i.e. through the identification of paternity and maternity) and it is now commonplace to distinguish between social and genetic mating systems. From the social point of view, mating systems refer to different strategies of obtaining mates, to different forms of pair bonding or mate guarding, which will depend on the spatial dispersion and temporal availability of the limiting sex (Emlen and Oring 1977; Grant *et al.* 1995). Genetic mating systems describe which individuals are reproducing and with whom (Hughes 1998). They often differ, for example when animals that form long-term pair bonds (social monogamy) produce extra-pair offspring (genetic polygamy) (Coltman *et al.* 1999a).

Until recently, estimates of male reproductive success have been based on behavioural indices (Dewsbury 1982). In polygynous animals, male mating success has traditionally been measured from the time during which a male has exclusive access to an oestrus females (e.g. for *Cervus elaphus*: Gibson and Guinness 1980), the dimension of the harem (e.g. for *Theropithecus gelada*: Dunbar 1984), from observed matings (e.g. for *Mirounga leonina*: Le Boeuf 1974; *Otaria byronia*: Campagna *et al.* 1988) or number of females guarded (e.g. *Panthera leo*: Packer *et al.* 1988). However, 'unconventional' and 'sneaky', less easy to observe mating tactics might exist (Hogg and Forbes 1997; Zamudio and Sinervo 2000). Alternative mating tactics might arise from different ways of achieving the same success or because not all individuals can successfully follow the best tactic. The payoff of any particular tactic may vary between individuals, for example between individuals of different quality. A common scenario is that success is unevenly distributed among individuals within a population, depending on body condition. In this case, some individuals may be more constrained than others in achieving the same success and hence adopt different tactics and make the best of their constrained situation (Cunningham and Birkhead 1998). Various elements can also reduce the reliability and the accuracy of behavioural estimates:

- females may copulate more than once and with different males during their oestrous period (Le Boeuf 1974);

- sperm competition might be present (Møller and Birkhead 1989) and female fertilisation dependent on mating order

- cryptic female choices might exist and show a female post-copulation control (Eberhard and Cordero 1995).

Molecular methods, therefore, offer the opportunity to test whether observed patterns of behaviour can be accurately translated into realised paternity. Combined with behavioural data, they provide a fundamental tool for the interpretation of mating system structure and evolution.

In a large number of apparently monogamous birds, extra-pair copulations represent a significant mating strategy and individuals show complex mating behaviours that greatly differ from those implied by socially monogamy (Burke *et al.* 1989). Molecular analyses showed that in indigo buntings (*Passerina cyanea*) extra-pair copulations were responsible for fertilising more than 14% of all offspring (Westneat 1987). In the emu (*Dromaius novaehollandiae*), the percentage of extra-pair copulations exceeded 50%, but sexual behaviour seemed to dynamically adapt to level of competition of mates at any time of the breeding season (Taylor *et al.* 2000). Studies on polygynous species have revealed widely different results. In some cases, dominance rank or copulation frequency reflected parentage (e.g. *Cervus elaphus*: Pemberton *et al.* 1992, Slate *et al.* 2000; *Gorilla gorilla beringei*: Robbins 1999). In these species, males usually guard groups of females, and there are relatively stable dominance hierarchies among males. Lower correlation may be present in species in which dominance relationships among males are less stable, where associations between females and males are brief and temporary, or where alternative tactics are possible (Coltman *et al.* 1999a,b). For example, males may follow alternative tactics in agamid lizards (*Ctenophorus ornatus*: LeBas 2001), Soay rams (*Ovis aries*: Coltman *et al.* 1999a), grey seals (*Halichoerus grypus*: Amos *et al.* 1993), and fur seals (*Arctocephalus gazella*: Gemmell *et al.* 2001). In this context, accurate parentage assignment allows determination of the 'genetic payoff' for observed behavioural strategies and calculation of both seasonal and lifetime reproductive success (Hughes 1998).

I.2 Site fidelity, dispersal and genetic structure

Dispersal is defined as the movement of an individual from its point of origin to the place where it reproduces or where it would have reproduced if it had survived and found a mate (Greenwood 1980). This differentiates dispersal events from feeding excursions.

Fidelity to breeding site (i.e. return to the same site to breed) and philopatry (i.e. return to the natal colony to breed) are common among mammals and they are thought to confer considerable reproductive advantages. Nevertheless, the pattern of fidelity and the extent of dispersal vary, depending on the reproductive system of the species and the sex of the individual. In the majority of polygynous mammals dispersal is sex-biased, and males disperse while females show site fidelity and philopatry (Greenwood 1980). Such gender differences in dispersal can influence the genetic structure of populations, particularly when the haploid and maternally inherited mitochondrial DNA is compared with the biparental nuclear genome. For example, significant differentiation in mtDNA but not in nuclear markers has been found between populations of fin whales (*Balenoptera physalus*: Bérubé *et al.* 1998), sperm whales (*Physeter macrocephalus*: Lyrholm *et al.* 1999) and white sharks (*Carchsrodon carcharias*: Pardini *et al.* 2001). In these cases, it was suggested that the variation was due to limited dispersal of females and extensive dispersal of males.

Some of the most striking examples of site fidelity also come from marine species, where highly mobile populations, in the presence of very few geographical barriers, can indeed be strongly structured. Each reproductive season, female green turtles (*Chelonya mydas*) migrate more than 2000 km from foraging grounds in Brazil to nesting sites on Ascension Island (Bowen *et al.* 1992). In this species, mitochondrial analysis found high structure both between oceans (Atlantic-Mediterranean vs Indo-Pacific) and breeding rockeries, identifying both geographic constraints (physical separation between oceans) and philopatric reproductive behaviour (natal homing within ocean) as the factors influencing the genetic structure of the species. Haplotype differences were also found between groups of humpback whales (*Megaptera novaeangliae*) that were following different patterns of migration – between summer feeding grounds in subpolar or temperate areas to winter breeding zones in the tropics (Baker *et al.* 1990). The authors interpreted the spatial segregation of genotypes as being the consequence of female fidelity to migratory destinations (Avise 1994).

Radio and satellite-tracking of pinnipeds have showed that seals and sea lions can travel many thousands of kilometres (Stewart and De Long 1994; McConnell and Fedak 1996; Stevick *et al.* 2002), but the extent to which individuals move between breeding sites is largely unknown. With such capability to travel long distances, one might expect interchanges between different breeding colonies.

However, site fidelity is largely present in pinnipeds, especially among females, and individuals usually return to breed at the same colony after long foraging trips.

I.2.1 Female gregariousness

In *Mirounga* and many otariids the level of female gregariousness is extreme during the breeding season. Theoretical predictions propose the concept of inclusive fitness (Hamilton 1964) to explain altruistic behaviours (i.e. behaviours that appear to benefit recipients instead of or more than the donor), though pinnipeds generally do not exhibit co-operative behaviour or social conditions that would suggest the operation of kin selection (Boness *et al.* 1993). Group breeding may involve significant costs to females, as it can facilitate parasite and disease transmission, can increase pup mortality and promote inter-female aggressiveness (Le Boeuf and Briggs 1977). However, it is clear that in these species selective factors contribute to the maintenance of the female tendency to aggregate. One of the benefits of breeding in a group or harem is the defence from male aggressive mating tactics, through the dilution effect of grouping and the exclusion of marginal males by higher-ranking males (Bartholomew 1970). Female elephant seals are much smaller than males, males have enlarged canines and they constantly show behavioural patterns of herding and biting while interacting with the females. Hence male-female interactions may be very risky for females. Female harassment is also a characteristic of other dimorphic pinnipeds (e.g. *Halichoerus grypus*: Boness *et al.* 1995; *Mirounga angustirostris*: Le Boeuf and Mesnick 1990a,b; *Otaria byronia*: Campagna *et al.* 1992) and it can involve serious injures for females and reduce their reproductive success (Le Boeuf and Mesnick 1990a; Boness *et al.* 1995).

Site fidelity and philopatry can also contribute to female aggregation. Females may return to the same site where they successfully bred the previous season, or to places where topographical features can benefit their breeding performance (Pomeroy *et al.* 2001). If females return to the same site to breed and aggregate in groups, the level of gene correlations might be higher within than between groups (Chesser 1991a) and might in turn lead to some advantages, including reduction of agonistic encounters or increase in likelihood of altruistic behaviours like fostering (Schaeff *et al.* 1999).

Female philopatry and concomitant male dispersal can result in differing degrees of genetic relatedness between male and female individuals within a population (Surridge *et al.* 1999). Similarly, it has been shown that polygyny coupled with female philopatry produces consistently high gene correlations among offspring and adults within social groups (Chesser 1991a). However, very few studies have investigated the pattern of genetic relatedness in polygynous (or lightly polygynous) species (*Oryctolagus cuniculus*: Surridge *et al.* 1999; *Phoca vitulina*: Schaeff *et al.* 1999; *Halichoerus grypus*: Pomeroy *et al.* 2000). In particular, genetic relatedness has yet to be investigated in a high polygynous species such as *Mirounga leonina*, where females tend to return to the same breeding colony each year and aggregate in large and defined harems during a short period of reproduction.

I.3 Background on *Mirounga leonina*: reproductive system and major life history traits

I.3.1 Mating system

Offshore marine feeding, terrestrial parturition and long fasting period have been identified as major factors in the evolution of pinniped mating systems (Bartholomew 1970). Many species are polygynous and sexually dimorphic; that is, males are much larger than females. Others are monogamous, or at least serially monogamous, in which case males and females are nearly the same size and look very much alike.

Pinnipeds have diverse mating systems and they can be divided into three categories according to breeding habitat (Riedman 1990): (1) those that breed on land, (2) those that breed on pack ice (floating ice), (3) those that breed on fast ice (ice attached to land). Of the 33 species of pinnipeds, 20 breed on land, while the remaining 13 breed on ice. A moderate to high degree of polygyny is found in all otariids, in the walrus and in only four phocids. Eighteen of the 20 species of land-breeding pinnipeds are polygynous and strictly sexually dimorphic, and breed in moderate-sized to extremely large colonies. Highly or moderately polygynous land breeding pinnipeds include all sea lions and fur seals and only three species of phocids: northern and southern elephant seals and grey seals. The remaining land breeders (which are probably polygynous to some degree) are the harbour seal and two species of monk seal. In contrast, 11 of the 13 species of ice-breeding seals appear to be either monogamous or serially monogamous, as well as monomorphic (the sexes are similar in size and appearance). The two ice breeders that do not fit the pattern, showing moderate polygyny, are the Weddell seal and the walrus. Except for the walruses, all of the ice breeders are phocid seals.

Many factors may account for this range of mating strategies: the breeding habitat and its ecological and climatic feature (Anderson *et al.* 1975; Le Boeuf 1991; Campagna and Le Boeuf 1988), the capacity for prolonged fasting, the temporal and spatial patterns of female distribution, the level of female gregariousness (Jouventin and Cornet 1980; Boness 1991) and the synchrony of female oestrus (Boyd 1991). In some species, males can control female movements and intensify their gregariousness by herding. Herding is widespread among pinnipeds but rarely effective. However, it is typical and effective in southern elephant seals. Harem holders frequently recruit arriving or solitary females to the their harems (Galimberti *et al.* 2000a), increasing the number of females in their harem and hence the potential reproductive resource to which they will have access. Their capacity of herding females is a major factor in defining the degree of polygyny of this mating system, and the individual male capability is the first requirement for a high level of mating monopolisation. Individual behavioural performance, coupled with the ability of fasting for months, allows protracted control of the females. Only a few males that are able to control matings during the entire breeding season will exhibit the highest reproductive success.

The breeding habitat can also have a strong effect in determining the level of pinniped polygyny, most of all because a reliable breeding environment encourages female gregariousness (Bartholomew 1970). Female gregariousness offers a high potential of mating monopolisation and sexual selection, providing the opportunity for some males to mate with many females. This results in intense competition among males for mates, which in turn leads to sexually dimorphic traits and polygyny. Among highly polygynous pinnipeds, males are much larger than females and have developed secondary sexual characteristics (armaments), such as the pendulous nose and frontal chest shield of the elephant seal or the hooded seal's balloon-like inflatable nasal sac.

Fig I.1 Size dimorphism between sexes and mating. a) a male southern elephant seal, age SAM4, with a medium-sized female. b) left: mating between an adult male and an oestrus female (often the male bites the female during the copulation to prevent her from moving); right: a peripheral male 'sneaks' in the harem and mates with a female while the harem holder is copulating and cannot see him

Southern elephant seals are extremely sexually dimorphic (Fig 1.1a) – adult males can be up to ten times larger than females (3000-4000 kg vs 400-900 kg; standard length of 4-5m vs 2.8 m (Laws 1994)) – and their mating system is thought to be among the purest forms of harem defence polygyny (McCann 1981, Le Boeuf and Reiter 1988). Males arrive on land first at the beginning of the breeding season. They fight and compete with each other, and the results of agonistic interactions set up a dominance hierarchy, so that hierarchy rank determines the breeding role of each male (McCann 1980; Galimberti *et al.* 2002c). One male (alpha or harem holder) has almost complete control of each female group and males not able to control a harem are kept outside the female groups as peripheral males. Females arrive ashore and typically stay on land for 27-28 days during the breeding season and aggregate in large groups (harems) of up to hundreds of females. They give birth within five days of their arrival and nurse their pup for approximately 23 days, before weaning it and then departing to the sea. They are in oestrus for the last two-three days of the nursing period (Campagna *et al.* 1993; Galimberti and Boitani 1999), and males attempt to copulate with them when they are either in the harem or departing from the colony (Fig I.1b).

Land breeding during a short reproductive season, a strong tendency of females to aggregate, extreme sexual dimorphism and low mobility are all factors that provide the potential for a high level of reproductive variance among males and for an extreme level of polygyny in southern elephant seals.

I.3.2 Pattern of growth and mortality

A given individual's pattern of mating arises from selective pressures under which that individual operates, and it will be directed by a combination of environmental, physiological and phylogenetic constraints. Moreover, the mating pattern of an individual is part of its life history, with the possibility of a large plasticity within the individual (as mating pattern and context can be different in different mating occasions) (Ahnesjö *et al.* 1993).

The potential for selection depends on the variation in reproductive success within sex, but the difference in phenotypic traits between males and females depends on the difference in selective pressure between them. Sexual selection is usually stronger in males than in females. As a consequence, phenotypic results of selective pressures differ between sexes (males are larger and show secondary sex characters). The main reason for the difference in sexual selection between sexes is that female reproduction is mainly limited by the number of offspring they can bear, while male reproduction is limited by the number of females that he can inseminate. Therefore, the number of offspring fathered by a male increases with the number of mates, whereas a female has less to gain from mating with several males, at least as concerns the number of her offspring. Consequently, phenotypic traits that make it easier to gain several mates would be favoured mainly in males (Andersson 1994). Male elephant seals differ from females in size, secondary sex traits, behaviour and level of aggressiveness, all traits that are fundamental for competing with other males and therefore for the access to the females. Moreover, their natural history significantly differs between sexes, and most of the differences are in growth, mortality rates and sexual maturity. Males mature sexually and begin breeding much later than females; they show higher mortality rates and have shorter life expectancy than females (McLaren 1993; Clinton and Le Boeuf 1993). Female growth rate is high in the first years and becomes almost steady when the female is four to five years old. Growth of males is a two-phase process, with a first increase in the first years, as for the females, and a large increase in growth rate just after puberty. Maturation in males covers a very long period (Fig I.2).

Puberty is reached when males are about five years old but true 'social maturity' is reached many years later, since they are usually not able to gain control of a harem until they are at least nine years old. By delaying reproduction and avoiding the risks and expenses in time and energy of reproductive effort, younger males may reduce the mortality associated with developing sexually selected traits, and thus increase their reproductive value (Clinton and Le Boeuf 1993). However, higher rate and longer period of growth, delayed maturity and larger size, together with competition for resources, social status and emigration, are male traits associated with increased mortality. Mortality of males is higher than for females (especially in the pre-breeding phase) (Fig I.3), and just a small percentage of each male cohort reaches full maturity and starts breeding (Le Boeuf and Reiter 1988).

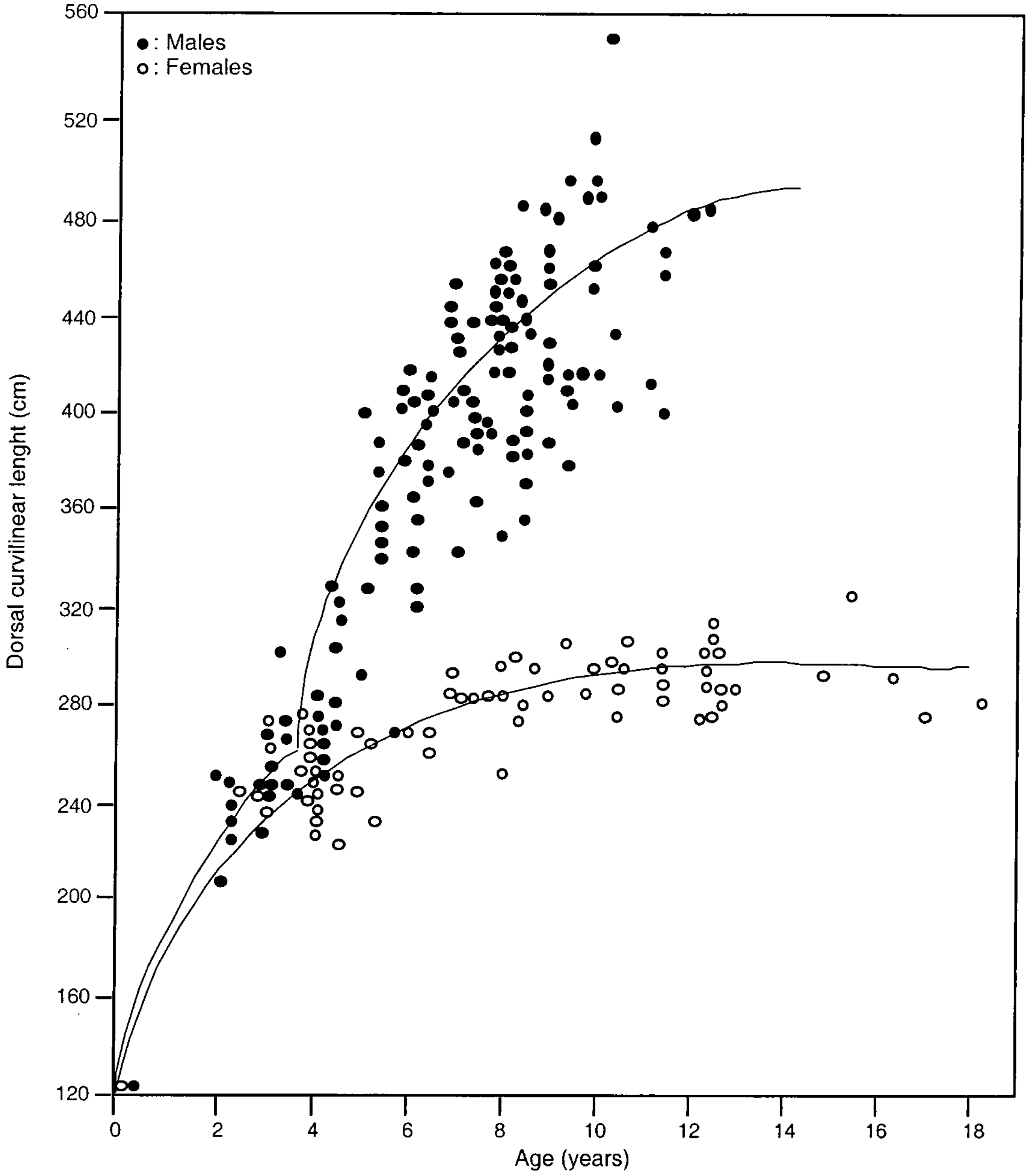

Fig I.2 Growth curves in male and female southern elephant seals. The growth patterns consistently differ between sexes. Around the fourth year of age, the curves strongly diverge and male growth shows a steep increase. Male growth increases throughout the maturation period, to slow down again when the male is 12–14 years old (from McLaren 1993)

Presumably, the evolutionary disadvantage of higher pre-reproductive mortality is paid off by the value of sexually selected traits used to attract mates. Small and immature males do not reproduce while the largest and most vigorous males will exhibit the highest reproductive success (Clinton and Le Boeuf 1993).

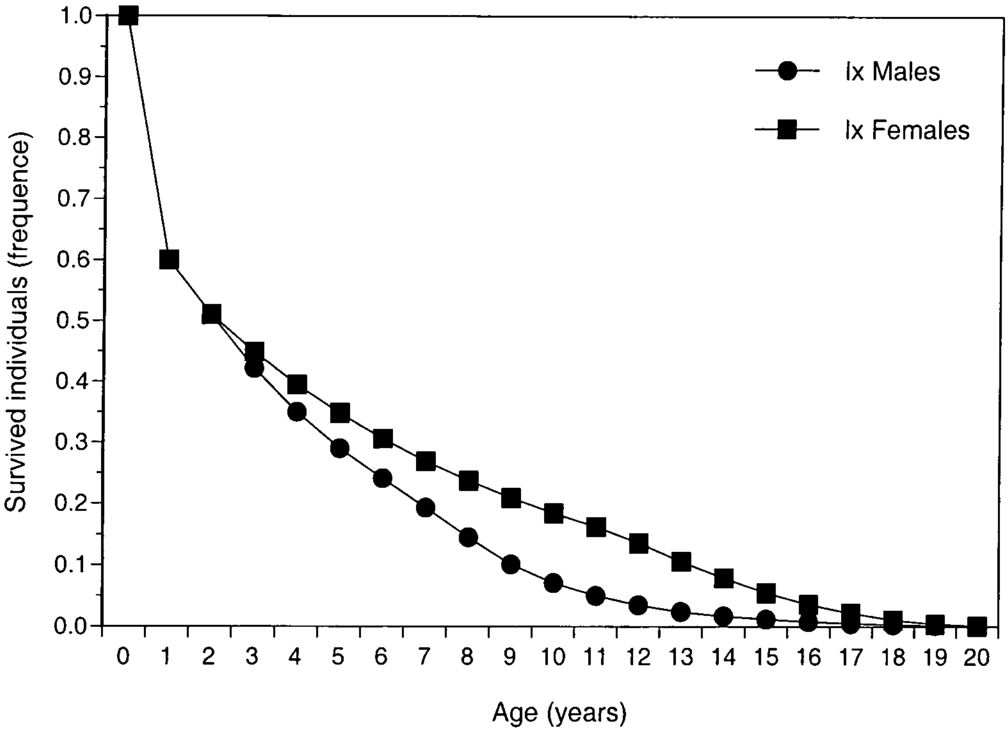

Fig I.3 Female and male survival curves in southern elephant seals. Mortality rates in this species differ between sexes. During the first two years, male mortality is similar to female mortality; after it becomes higher and males lives shorter lives (adapted from McCann 1985)

I.4 Objectives

The general objective of this research is the analysis of the genetic structure of the southern elephant seal (*Mirounga leonina*). In particular, the mating system of the species is investigated from a genetic perspective, molecular results are compared with behavioural data, and individual genotype distributions are analysed within and between breeding populations.

The different subjects are presented in the following chapters:

1) Male reproductive success: behavioural estimates and paternity

Elephant seals are among the most sexually dimorphic and polygynous species of all mammals. Results from behavioural studies indicate extreme monopolisation of matings by only a few males each breeding season; consequently, an extreme variance in paternity success is expected. High variance in reproductive success can be an indicator of past sexual selection and a potential source for current sexual selection. Hence, a good estimation of individual success is fundamental in understanding mating system evolution and, in this context, elephant seals represent an ideal study species.

This chapter presents the distribution of genetic paternity in the Sea Lion Island's population of elephant seals across two consecutive breeding seasons, and quantifies the level of polygyny and male reproductive variance in the population. It describes the reproductive success of males from seven different harems, corresponding to seven independent breeding contexts. Finally, it compares genetic results with behavioural estimates of male mating success, in order to define the effectiveness of behavioural indices in predicting paternity.

2) Patterns of relatedness within the colony

Southern elephant seals are a highly gregarious species during the breeding season, and site fidelity is present among both females and males. Social structuring and site fidelity may lead to non-random mating patterns within and between groups, and provide an additional level at which genetic variation may be maintained. Nevertheless, patterns of relatedness have yet to be investigated in this species.

This chapter presents data on the distribution of genetic relatedness in the population of elephant seals of Sea Lion Island. First, the accuracy of the genetic estimates is determined from the analysis of known relationships. Following this, the patterns of kinship among seals are assessed to investigate the presence of any genetic pattern influencing the social structure of the population, and to test whether site fidelity and philopatry are good predictors of relatedness.

3) Population genetic structure

In polygynous species, variance in male reproductive success may alter the genotypic variation from that expected with panmixia. It will reduce N_e within local populations and hence local genetic diversity. In the absence of genetic dispersal among colonies, local populations could highly differentiate among each other and within population variation further decrease. Genetic differences among southern elephant seal breeding colonies have been previously detected, with most of the geographic structure due to the divergent Macquarie and Peninsula Valdés populations. Pronounced within-stock variation has been found between the populations of Peninsula Valdés and South Georgia. However, data from the geographically intermediate Falklands have never been included in any population comparison.

This chapter quantifies the genetic variation in the populations of Sea Lion Island (Falklands) and Elephant Island (South Shetlands), and investigates it in the context of previous results from other colonies of the same South Georgia stock and from the more distant Macquarie and Heard Islands. Finally, with the comparison of mitochondrial and nuclear markers, it considers male and female patterns of dispersal.

II. Materials and Methods

II.1 The study population and field site

The fieldwork was carried out on Sea Lion Island (52°26' S; 59°05' W) during three breeding seasons (September-November, from 1996 to 1998). Sea Lion Island is the southernmost island of the Falklands and the main breeding site for southern elephant seals in the archipelago (Fig II.1).

The population on Sea Lion Island (SLI hereafter) is small and localised (i.e. with no other breeding sites close to the island), estimated to be around 1820 seals of one or more years of age (Galimberti and Sanvito 2001). During the study, the population increased from 527 breeding females in 1996 to 567 in 1998.

II.1.1 Topography and spatial distribution

The study area covered the eastern point of the island. The coastline is composed of three continuous stretches of sandy beaches divided by a rocky area; breeding units occupied only sandy beaches (total length ca = 4.4 km, estimated from aerial photographs). *Harems* were scattered along the beaches and their spacing was uneven, with long stretches of beach with no females (Fig II.2).

The mean density of females was 111 females per km of coastline suitable for breeding, and eleven harems were formed in 1996 and twelve in 1997 and 1998. There was extensive variation in the harem size within each season and among years (for the three seasons: median = 35 females; median absolute deviation, *MAD* = 25; Min = 4; Max = 119).

Since detailed maps of the island were not available, to have topographical reference in locating individuals and harems, the whole study area was divided into different *zones* and *areas* (see below for definitions) using topographical landmarks. The position of each landmark was identified using GPS receivers with differential post-processing (precision < 3 meters RMS: Magellan System Corporation 1995. Professional products: operation manual. San Dimas, CA) and located on a map drawn from aerial photography (RAF Mount Pleasant Airport, Falkland Islands).

The same area division and nomenclature were used each year by each observer and this gave the possibility to identify the position of harems and individuals in detail.

II.1.2 Relevant definitions

II.1.2.1 General

- *Breeding season*: a 12-week period (beginning of September – end of November) that included the arrival of the first breeding males and the return to sea of the last breeding females. This period was centred on the *peak haul out*, the day on which the maximum number of females on land was counted. The day is almost constant across years (20th and 19th of October – see also Galimberti and Boitani 1999).
- *Harem*: a group of two or more females with a male in attendance (Fig II.3). Females were considered grouped when their distance apart was less than or equal to 10 standard body lengths (SBL – American Society of Mammalogists 1967). Each day, *harem size* was defined as the number of females in the harem based on daily counts. Over the season, *harem size* was the maximum size reached over the censuses the season (and different harems may reach maximum size on different days).
- *Breeding area*: a continuous stretch of beach, separated from other *areas* by evident landmarks (recorded with GPS, see above) and not by unsuitable breeding habitat for elephant seals (rocks or cliffs). *Breeding zone*, on the contrary, was a stretch of beach separated from other *zones* by stretches of habitat not suitable for elephant seals and not used for breeding. Areas and zones were delimited by the same fixed landmarks each year; each *area* may include one or more harem*s* while each *zone* comprised two or more *areas*. The study area was divided into three zones: STRE (with 7 breeding areas), GENT (with 4 breeding areas), DUNE (with 2 breeding areas) (see Fig II.2).
- *Breeding male*: any male present in the breeding areas under daily observations and classified as behaviourally active in reproduction.
- *Breeding female*: any female that belonged to a harem and produced a pup during the season.

Fig II.1 Position of the Falklands and of Sea Lion Island. The study area on the island is also showed

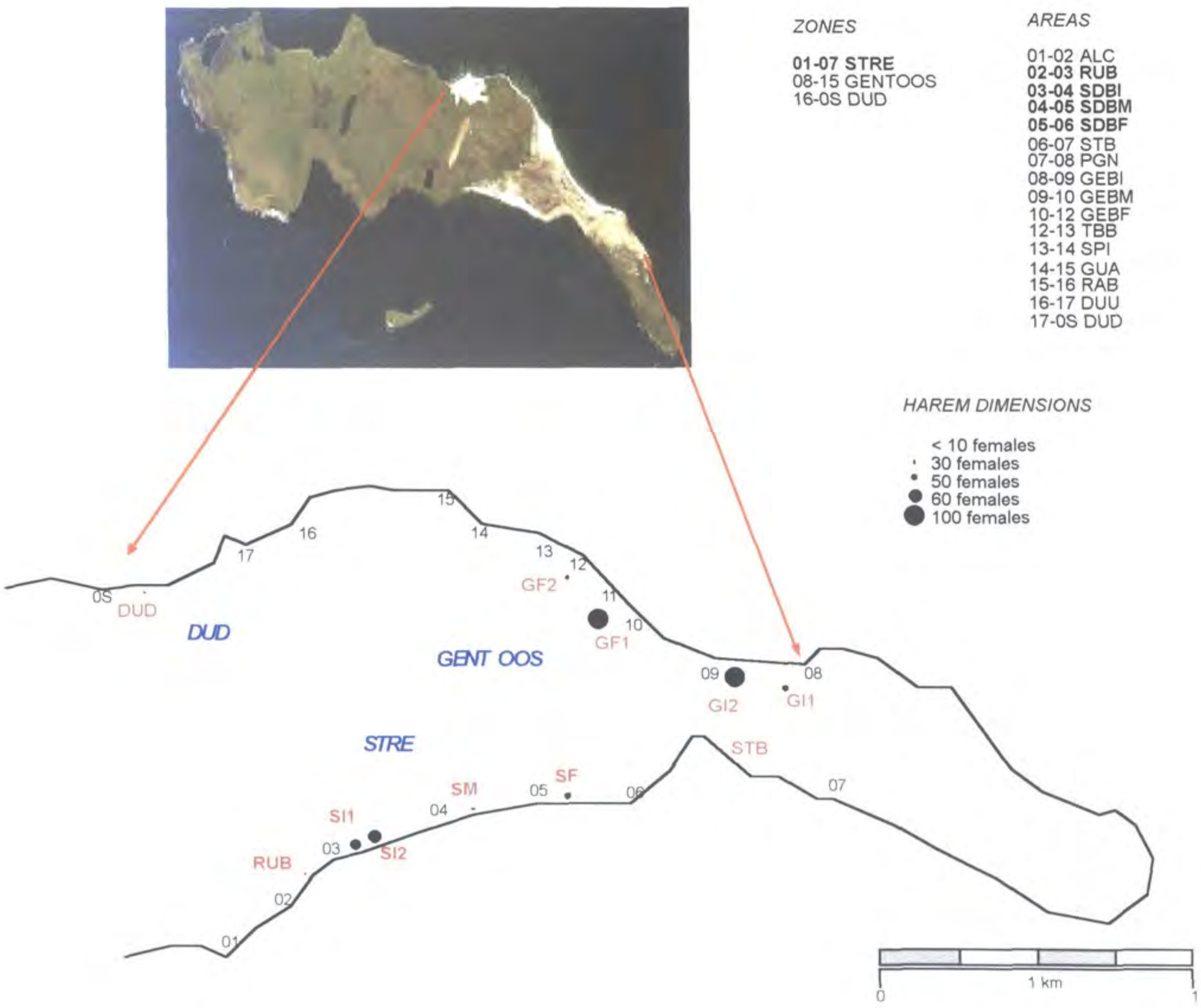

Fig II.2 Distribution and dimension of the *harems* (black dots) in 1996. *Harem* names are indicated in red. Numbers on the map defines *areas* (list on the right) while *zone* names are in blue (list on the left). The *harems*, *areas* and *zone* of which genetic data were analysed are indicated in bold either in the lists or on the map

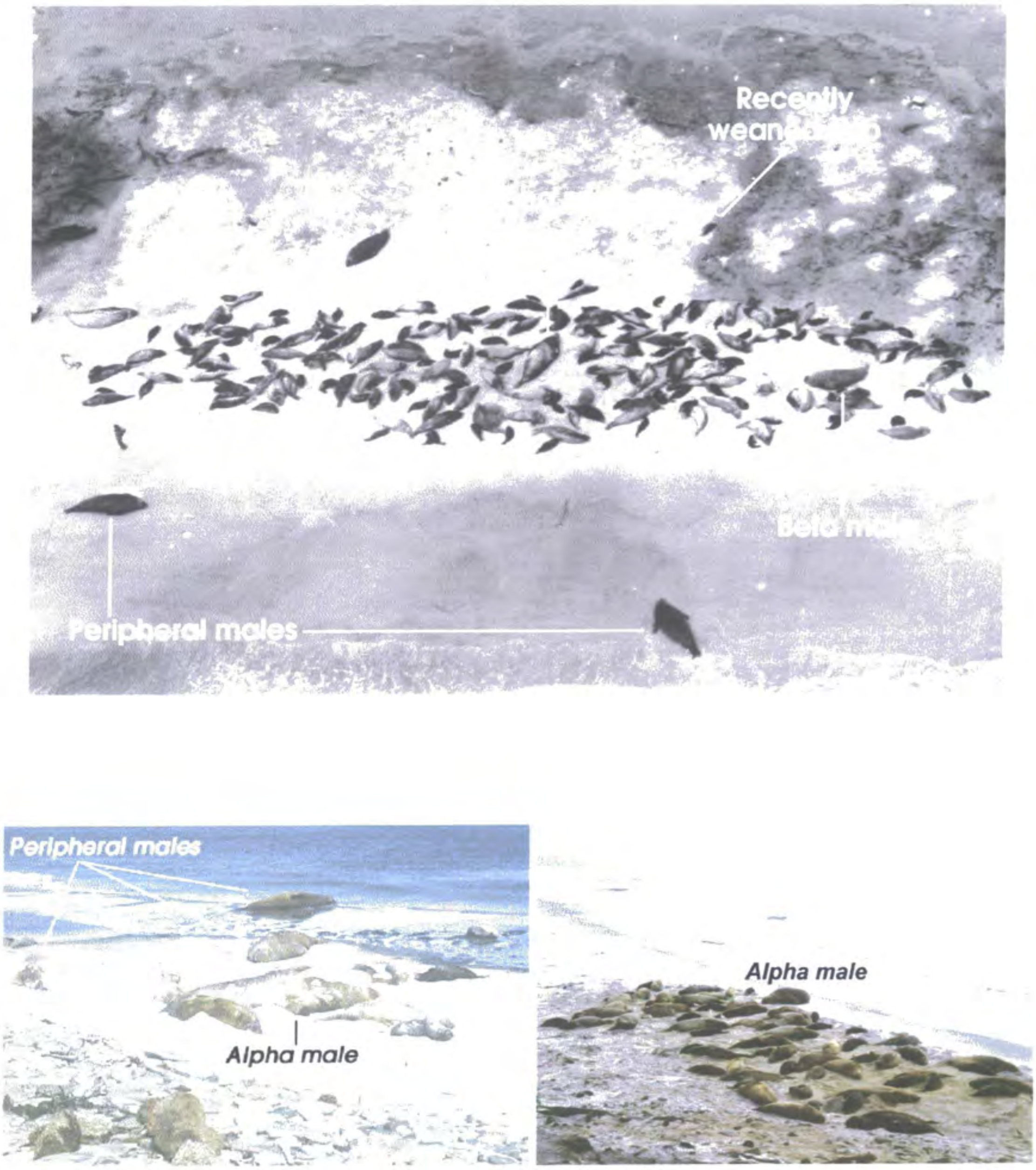

Fig II.3 Different harems on SLI.The harems have different size (number of females) and different number of males associated. In each harem the alpha male, peripheral and beta males (when present) are indicated

II.1.2.2 Male traits

- *Age classes*: males were classified into age classes. Size was used to recognise gross differences, but finer classification was achieved using external morphology (spread of scars on the frontal shield and neck, appearance of the facial area and development of proboscis) in order to obtain an estimate as independent from size as possible. Eight age classes were recognised: yearling (*Y*) estimated age 1 year; estimated age 2 years (*2Y*); juveniles (*JUV*), age 3 years; sub-adults (*SAM1* to *SAM4*), age from age 4 to 7 years; adults (*AD*), estimated age 8 yr or more (see Galimberti and Boitani 1999 for consistency of the classification among different observers).
- *Tenure*: the period that a male stayed on land for reproduction.
- *Daily breeding status*: male status was recorded during daily censuses and the categories defined were based on the distance of the male from the closest female group. Standard female body length (ca. 2.6 m) was estimated by eye as an approximate measure of distance between individuals (Baldi *et al.* 1996) and used to define the daily status of each male. Five categories were recognised: *harem holder* (*or alpha*), if the male was in the harem (0 distance), *beta* if he was inside the female group but with less females on his side than the harem holder; *peripheral*, if 1 to 5 SBL distant from the most external female of the harem; *marginal*, if 6 to 10 SBL distant; *solitary*, if more than 10 SBL distant.
- *Seasonal breeding status*: males were classified as *principal males* (or *harem holders*) if they gained control of a harem for more than 24 hours (at least two consecutive censuses); *secondary*, if they never got control of a harem for more than 24 hours but they were associated to a harem (as *peripheral* or *marginal* males); *tertiary*, if they were never observed associated to a harem. If more than one male during the breeding season controlled a harem, the male that held it for the longest period was defined as the *seasonal holder*, while the others were defined as *temporary holders* (usually keeping the control of the females for short periods).
- *Opportunity of selection* (*I*): an estimate of variability of success, denoted by *I*. It is calculated as the variance in mating or reproductive success divided by the square of the mean ($s^2/\bar{x}$), following Arnold and Wade (1984). It is a measure of the potential of selection, and it has been proposed as an index of the extent of variation that could

be subject to selection in a given group, population or species (i.e. it describes the upper limit on changes in phenotype that may be caused by selection or variation in each analysed system). I is proportional to the variance in reproductive success and its magnitude would indicate the potential for selection: small values would indicate little opportunity for selection, whereas large values would indicate greater opportunity for selection. Since I has no units, it has been used to make comparisons between sexes and species (Clutton-Brock 1988). Such comparisons may allow making inference regarding the relationship between opportunities of selection and mating systems, as increased opportunity for selection imply a greater potential for evolutionary change (Downhover *et al.* 1987). In this study, it has been used to describe and quantify the variance in male mating success and paternity. A truly monogamous mating system will show an opportunity of selection of zero, while a polygynous system will exhibit a larger value (i.e. 0.5-1.7 for red bishops, Friedl and Klump 1999; 0.56 for grey seals, Anderson *et al.* 1975; 1.31 for southern sea lions, Galimberti unpublished data; 2-3 for red deer, Clutton-Brock *et al.* 1988).

II.2 Field work and behavioural methods

II.2.1 Population marking and censing

All males and almost all females (>98% each season) were individually approached and marked. Seals were at least double tagged in the interdigital membrane of the hind flippers using nylon cattle tags (Jumbo Rototags™, Dalton Supplies Ltd – Fig II.4). Most breeding males were marked with three or four tags to increase the likelihood of recognition in following years. Tag-loss rate was very low among all seasons: in 1997 and 1998 the likelihood of loosing both tags, as calculated from a binomial model applied to double-tagged individuals, was 0.0031 for both males and females (Galimberti and Sanvito 2000). All males and the majority of females (80-85%) were also marked by painting an identification code on their flanks and back using commercial black hair dye (Fig II.5). The paint marks lasted for the entire breeding season and until the moult. The joint use of tags and paint marks ensured recognition of all breeding animals.

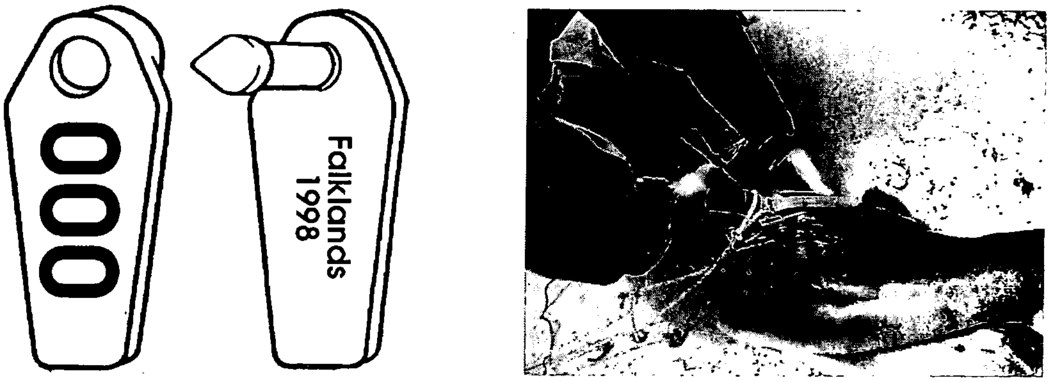

Fig II.4 Left: Jumbo Rototag with a 3 digit numerical code; right: the tag is applied on a weaned pup, in the membrane of his hind flipper

The tagging also allowed an accurate estimate of the net production of breeding, all pups were marked with one tag when they were still with their mothers and then again after weaning.

On each of the 84 days of the breeding season, all seals were counted by walking along all breeding beaches during low tide. From daily censuses three kinds of data were obtained:

- counts of the number of individuals on land by sex and age class;

- data on the structure of the harems (number, identity and breeding status of females; number, breeding status and position of alpha and associated males);

- data on breeding status and position of males and females not associated with any harem.

To check for the presence of breeding individuals outside the main study area, weekly censuses were also taken by walking the entire perimeter of the island. Only two pups were born outside the study area during the three breeding seasons.

Fig II.5 Female and SAM4 male with the identity name painted on their side with black hair dye

II.2.2 Behavioural observations

Each season four people carried out observations of the seal population. To gather data on breeding activities, each observer recorded copulations (see Fig I.1b), arrivals on land, departures to sea, births and weaning events (departure of the mother and subsequent expulsion of the pup from the harem). Data were collected by each person during two-hour periods from fixed observation points overlooking one or more harems. A standard 'all-occurrence' sampling technique was used (Altmann 1974) with continuous recording of events on log sheets. During the observation periods, all male-male and male-female interactions (aggressive and mating interactions) in the harems were recorded. Behavioural events were coded using a standard ethogram (Fabiani 1996), analogous to the one described for the northern elephant seal (Le Boeuf 1972 1974). The majority of observations were carried out between 0600 and 2000 local time, but a limited number of night observations were also conducted, using spot lights and night-viewing equipment. Since no difference in seal activity was found between day and night (see also Baldi *et al.* 1996, and Shipley and Strecker 1986 for the northern species), the observational effort was concentrated during day-light.

The total number of hours of observation was 1,316 in 1996 and 1,156 in 1997. To collect data in different breeding situations, all harems of the study area were observed. Observational effort was balanced among the harems, except for small harems that lasted for only short periods. Ten harems formed in 1996 and 11 in 1997; a mean of 131.6 ± 68 hrs of observation per harem were made in the first year and of 105.5 ± 48 in the second.

For genetic analysis, one zone (STRE) of the whole study area was chosen and the observational data used were from five harems for 1996 and two for 1997. For the seven harems, a total of 1,030 hrs of observation were conducted and 356 copulations recorded (means per harem 147 and 51, in 1996 and 1997 respectively). Males were very rarely seen moving among zones: movements of peripheral males were occasional and almost zero for alpha males. By constrast, harems belonging to the same breeding area were often connected through the movement of peripheral and marginal males. Occasionally alphas moved between harems in the same area and less frequently between areas.

II.2.3 Sample collection

Every season skin samples were collected from females belonging to the harems of the STRE zone, all pups and all breeding males. Samples were taken from the interdigital web of the hind flipper of each animal, using ear-punch pliers (Fig II.6).

Fig II.6 Skin sampling: a skin sample is obtained from the interdigital membrane of male elephant seal

The sampled skins were put in 1.5 ml microfuge tubes and preserved in the field in 100% EtOH, following the protocol suggested by Dessauer *et al.* (1990). Sampling of mothers and putative fathers during one season was coupled with sampling of pups born during the next season. So there were complete series of samples for 1996 and 1997. In total, in 1996 and 1997, samples were taken from females belonging to 16 harems (ranging in size from 4 to 91 females) for a total of 446 sampled females. Samples of males for the same seasons were 122; samples from all pups born in 1997 and 1998 were 1067.

For the paternity analysis, only the females sampled in the seven harems of the STRE zone and their respective pups from the next season were considered (five harems for 1996 and two for 1997). All breeding males of the 1996 and 1997 breeding seasons were included in the paternity analysis. The sampling covered 95% of males for both years and 50 to 80 percent of the females that bred in each harem.

II.3 Behavioural and genetic analysis

II.3.Indices of male breeding performance

Three indices of male breeding performance were estimated from behavioural data:

- an index of female control (*FFD*), calculated as the sum of the females controlled each day by a male throughout the breeding season (see "females/days index": Clutton-Brock *et al.* 1982);

- an index of mating success (*MS100*), calculated as the number of copulations by a male in 100 hours of observation (Fabiani 1996). Copulations were considered successful only if intromission lasted more than 60 seconds.

- an index of fertilization success (*ENFI*), calculated as the product between the proportion of copulations achieved by a male in one harem and the number of females that bred in that harem, summed over harems in which the male copulated (Le Boeuf 1974).

II.3.2 Statistics used

Behavioural and genetic data from both years were pooled together in most cases, after checking for homogeneity with a Mann-Whitney test. Paternity inference was, instead, carried out separately on each year. The coefficient of determination (R^2) was used as measure of effectiveness of mating estimates in predicting paternity (Pemberton *et al.* 1992; Coltman *et al.* 1999a). It represents the proportion of the dependent variable's variability that is explained by the independent variable (with a maximum value of 1; thus, an R^2 of 0.80 means that 80% of the dependent variable's variation is explained by the independent variable).

Statistics are presented as mean and standard deviation (*sd*), or median (*med*) and median absolute deviation (*MAD*) for asymmetrically distributed variables. The *MAD* is a measure of variability (or spread) analogous to the standard deviation. As standard deviation averages the variability of actual points from the mean, *MAD* takes the median of differences between points and the median; as median is less vulnerable to extreme data points than the mean, *MAD* is less vulnerable to outliers than standard deviation. The coefficient of variation (*CV*) is presented to describe the variability of the distributions, and the skewness value (g_1) to show the level of asymmetry. Due to the high frequency of asymmetric distributions, non-parametric tests were mostly used. Parametric and non-parametric tests were run in StatView 5.0 (SAS Institute). Non-parametric randomisation tests were employed in StatXact Turbo 4.0 (Cytel Software Corporation), and in these cases the *P* value is shown with Monte Carlo resampling number (as subscript). Probability levels and 95% confidence limits for the coefficients of the determination were calculated with the R2 software (Steiger and Fouladi 1992 – available at: http://www.interchg.ubc.ca/steiger/r2.htm).

In case of tests with multiple comparisons, since simple Bonferroni correction is known to be very conservative, the sequential method of Holm (1979)– also known as ‘sequential Bonferroni’– was applied. The test was used to avoid losing too much statistical power and it was implemented in the Multiplicity Program 2 (Brown and Russell 1996).

II.4. Molecular genetic methods

II.4.1 DNA extraction and visualization

The same protocol of Phenol/Chloroform extraction (Hoelzel 1998) was followed for all samples. A small piece of skin without fat (2-3 millimetres width) was put in a 1.5 ml microcentrifuge tube with 500 µl of Digestion Buffer (100 mM NaCl, 10 mM Tris-HCl pH8.0, 20mM EDTA) and chopped for few minutes until reduced to almost powder-size pieces. Thirty µl of proteinase K solution (10 mg/ml) was added, the tube mixed gently for 1 min and incubated at 37°C overnight. Five hundred µl of phenol was added to the mixture, the tube shaken moderately and centrifuged for one min at 13,000 rpm (Hettic Zentrifugen - EBA 12). The aqueous phase was collected, transferred into a new microfuge tube and 500 µl of phenol:chloroform:isoamyl alcohol (25:24:1) was added. The tube was shaken and centrifuged for 1 min. The aqueous phase was collected, transferred into a new microfuge tube and 500 µl of chloroform:isoamyl alcohol (24:1) was added. The tube was shaken and centrifuged for 1 min. The aqueous phase was again collected and mixed with 50 µl of 3M sodium acetate plus 500 µl of cold 100% ethanol. The tube was gently mixed and placed in the freezer for one hour (or left at room temperature for 20 min if any precipitate was visible). The sample was centrifuged for 10 - 15 min and the supernatant discarded (or the pellet removed with a sterile pipette). The pellet was washed in 1 ml of 70% ethanol, mixed and centrifuged for 10 min. The supernatant was discarded as before and the pellet dried under vacuum. The pellet was dissolved in 500 µl of TE, or similar volume, to a final concentration of about 200 ng/µl. The sample was in a bath at 65°C for 10 - 15 minutes and stored at -20°C.

In total, 455 samples were extracted from the SLI population: 101 males, 162 females and 192 pups. Thirty-nine males and 30 females were present on the island in both years. Thirty samples were also extracted from the population of Elephant Island (EI), following the same procedure as for SLI individuals.

The extracted samples were visualised to estimate the presence and state of the DNA. Electrophoresis of DNA samples was performed using 0.8 - 1.2 % agarose 0.5 x TBE (Tris-borate, EDTA) gels, with 0.5 x TBE as buffer. Two drops of a 50 µg/ml solution of ethidium bromide was added to the molten gel to allow DNA to be visualized. DNA samples were prepared as: 2 µl of loading buffer (30% glycerol, 0.25% bromophenol blue, O.25% xylene cyanol FF), 5 µl of H_20 and 5 µl of DNA. Gels were run horizontally (Bio Rad - Wide Mini-Sub™ Cell, Mini-Sub Cell GT) at 50 - 100 V and after visualised on Bio Rad gel Doc 2000 and analysed with the software Quantity One 4.0.3. All the extracted samples (SLI and EI populations) were visualized on gel.

II.4.2 Microsatellite DNA

II.4.2.1 Microsatellite markers for PCR amplification

Published microsatellite primers known to amplify for phocid species (Coltman *et al.* 1996, Gemmell *et al.* 1997; Goodman 1997a; Slade *et al.* 1998; Hoelzel *et al.* 1999) were used. More than twenty sets of primers were tested by carrying out PCR amplifications on the samples, so that the optimal conditions (annealing temperature and magnesium concentrations) were found. Eight sets of primers were chosen out of the 14 sets screened. These loci consisted of four isolated from grey seals *Halicoerus Grypus* (Hg4.2, Hg6.3, Hg8.9, Hg8.10), one from the harbour seal *Phoca vitulina* (Pv9) and three from southern elephant seal *Mirounga leonina* (BETA, M11a, M2b). The eight primers showed clear amplification products and high variation, and provided sufficient resolution to permit paternity testing and an assessment of population genetic structure (Table II.1). Except for BETA, they all amplified dinucleotide repeat sequences (size 130 to 340 bp) and for one locus. BETA amplified pentanucleotide repeats $(GGAAA)_n$ and for two loci (four alleles). Multiple alleles at a given size could be detected by the height of the peak, but single-locus genotypes could not be determined (Slade *et al.* 1998). These two related loci were treated as a single locus with four alleles and used separately from the other microsatellites in the analysis.

II.4.2.2 PCR (Polymerase Chain Reaction)

Each PCR amplification (10 - 20 µl) was performed following standard conditions: 0.2mM $dNTP_s$, 0.75 - 1.5 mM $MgCl_2$, 10 mM Tris-HCl PH8.4, 500mM KCl (Hoelzel and Green 1998), 0.02U/µl Taq polymerase, 250 pM, 150 - 250pM of each primer, 5 - 50 ngµl of DNA. PCR reactions involved the following steps: 5 minutes of denaturing at 95 °C; a cycle repeated 34 times consisting of: 1 min 30 sec of the annealing temperature (specific for each primer), 1 min 30 sec at 72°C extension temperature and 45 sec at 94°C; then 1 min 30 sec at the annealing temperature and 8 min at 72°C. The samples were then kept in the machine at 4 - 8 °C. The primer BETA had different PCR buffer and amplification. The buffer used was 500mM KCl, 100mM Tris Ph8, Nonidet P-40, Tween 20, 1.5mM $MgCl_2$. Cycling parameters: 94°C for 5 min, then 40 sec at variable annealing temperatures,

Primer	Primer sequence	Ann. T (¡C)	$MgCl_2$ (mM)	allele size (bp)	number alleles
BETA/	F: AAT TAG TAT GAT GCT GGG CTG TC	67,66,65	1.25	269-339	15
	R: AAT TGG GCA TGT GAT GTG ATG AG				
Hg4.2 *	F: AAT CGA AAT GCT GAG CCT CC	57	1.0	135-141	4
	R: TGA TTT GAC TTC CCT TCC CTG				
Hg6.3 *	F: CAG GGG ACC TGA GTG CTT ATG	55	0.75	215-225	6
	R: GAC CCA GCA TCA GAA CTC AAG				
Hg8.10 *	F: TGT TAA CTA TCT GGC ACA GAG TAA G	55	0.75	178-191	9
	R: TTT CCT ATG GGT TCT ACT CTC				
Hg8.9 *	F: AAT TCT GAA GCA GCC CAA G	55	1.25	175-195	9
	R: GAA TTC TTT TCT AGC ATA GGT TG				
M11a ^	F: TGT TTC CCA GTT TTA CCA	51	1.0	141-151	6
	R: TAC ATT CAC AAG GCT CAA				
M2b^	F: CCG ACT GCT GGG GTA AAG	60	1.5	235-255	10
	R: TCA GTC TCA CCC ACC TAC				
PV9 *	F: TAG TGT TTG GAA ATG AGT TGG CA	55		162-170	4
	R: ACT GAT CCT TGT GAA TCC CAG C				

Table II.1 Sequences for the 9 loci analysed, their annealing temperature, allele size, number of alleles, observed (H_0) and expected (H_E) heterozigosity, null allele frequencies. Sequences from: * Allen et al. (1995),/Slade et al (1998), ^ Hoelzel et al (2001) extension at 72° for 2 min and 94°C for 45 sec. The annealing temperatures followed a "touchdown" procedure to reduce non-specific amplification and were 67°C for the first cycle, 66°C for the second cycle, and then 65°C for 25 cycles. The amplifications were all performed on one of the following PCR machines: MJ Research, INC-PTC-100™, Hybaid - PCREXPRESS™, Perkin Elmer – GeneAmp PCR system 2400™.

To visualize the product on a sequencing gel on the automated ABI system, fluorescently labelled primers were used. The forward sequence of each primer was labelled with 6-FAM, HEX, NED ABI dyes (each one visualizing a different colour) and PCRs were run using 1/10th of fluorescent primer and 9/10th unlabelled forward primer. The cycling conditions were the same as above.

All samples were amplified for all loci and visualized on 0.8 - 1.2% agarose gel (see above for gel preparation), to verify the quality of the amplification. If no product was visible the sample was amplified again and PCR conditions adjusted as necessary.

II.4.2.3 Microsatellite allele detection

The labelled PCR products were run on a sequencing 6% polyacrilammide denaturing gel and analysed for length variation on an automated ABI PRISM 377 DNA Sequencer (Applied Biosystems™). The different dyes have different strengths, so the volume of PCR product to be loaded on the gel varied with the dye. I used 0.2 μl for 6-FAM-labelled products, 0.3 μl for HEX and 0.4 μl for NED-labelled. PCR products with sizes that did not overlap or products with different colours could be run in the same lane of a gel. In general, 7 to 10 primers were run in each lane for 36-50 lanes. The gels were run at 41°C for 3 hours on an automated sequencing machine, using filter set D. The dye ROX-500 was used as an internal size standard: it is loaded in the same lane as the PCR products and it serves to give precise band sizing between lanes.

Data from the gels were analysed using GeneScan™ Analysis 2.0 software. The computer generates a gel image showing bands that are detected as peaks of different colours, depending on the fluorescent used. The ROX-500 contains products with peaks at 35, 50, 70, 100, 139, 150, 160, 200, 250, 300, 350, 400, 450, 490, 500 bp. When using filter set D, 6-FAM appears blue, HEX green, NED yellow and ROX is red.

Each allele can be assigned an estimated size based on its migration relative to the size standard so that DNA allele sizes (in base pairs) can be precisely estimated. The data from the laser are collected in terms of number of scans that had been made when peaks are detected. The internal size standard is used to convert number of scans into base pairs within each lane in order to allow for lane to lane variation which can occur when gels do not run straight. The software program recognises the standard peaks and constructs a standard curve. The sizes of the product peaks are then estimated based on their migration relative to the known standard.

The data were then transferred to the ABI software package, Genotyper™ 2.0. With this program all run lanes can be viewed together on an electropherogram, and each detectable peak with its colour and its size can be scored in base pairs. In the electropherograms, the allele peaks often have reduced 'stutter' or 'shadow' bands (David and Menotti-Raymond 1998) of variable magnitudes. The allele sizes were easy to score the majority of the time; otherwise the sample was run again on another gel. The allele sizes shown by the program are not integers and there can be variations within and among gels of up to 0.6 base pair. The allele sizes were therefore classified into allele categories, each one expressing the size as an integer number. Because identical alleles can show little shifting in size across multiple gels, a 'control sample' (a sample of known size and previously run) was included on each gel in order to confirm that the allele size estimation was consistent among gels.

II.4.3 Mt DNA

II.4.3.1 MtDNA amplification

The mitochondrial DNA control region was amplified with universal primers, MTCRf (5' - TTC CCC GGT GTA AAC C) and MTCRr (5' - ATT TTC AGT GTC TTG CTT T) following Hoelzel *et al.* (1993). PCR reaction conditions: 0.2 dNTPs, 1.5 mM MgC12, 10mM Tris-HCl Ph8.4, 50mM KCl, 200 - 250pM of each primer, 0.02 U/µl Taq polymerase and 3 - 6ng/µl of DNA. The amplification involved the steps: 4 minutes of denaturation at 94°C and 35 cycles of 1 min and 30 sec at 50°C (annealing temperature), 1 min and 30 sec at 72°C, 45 sec at 90°C.

II.4.3.2 MtDNA sequencing and alignment

PCR products were then purified with QIAgen PCR purification columns, and sequenced directly using the dye-terminator method for the ABI system. The DNA sequencing reactions were performed using standard conditions: 25 cycles of 10 sec at 96°C, 7 sec at 50°C and 4 min at 60°C. MtDNA from 57 individuals from Sea Lion Island (30 males and 27 females) was amplified and the amplifications resulted in 450 – 650 bp of sequence data. The analysis focussed on a highly variable 299 bp subset, comprising all 264 bp of control region I (CRI) and 35 bp of flanking tRNApro sequence (Slade *et al.* 1998).

The sequenced products were run on a 6% denaturing polyacrilammide DNA sequencing gel for fluorescent imagining on an automated ABI PRISM 377 DNA sequencer. At the end of the sequence run, computer files are created for individual lane scans and a DNA sequence file is generated. In each file both peaks at each position and the nucleotide assigned by the instrument are defined. The sequence files were then imported into the program Sequencher 3.0™ that allows viewing multiple electropherograms simultaneously and a detailed checking. For each sequence it displays the electropherogram as well as the DNA sequence, making it possible to view regions of overlap, to look at the actual peak, and manually solve sequence ambiguities or change nucleotides mistakenly assigned by the sequencer machine. After the sequence formatting, the sequences were aligned in Sequencher 3.0™ and a Consensus file was generated as input for the phylogenetic analysis.

To confirm the sequencing accuracy, a reverse amplification and sequencing were run. To avoid sequencing the hypervariable region (Hoelzel *et al.* 1993b), a new R primer was developed (5' GTA TGA TGT TGG TAA ATG A 3'). The sequenced products (6 samples were sequenced) confirmed the previous results.

The mitochondrial DNA analysis also covered other populations of the South Atlantic. Thirty elephant seals from Elephant Island (EI) were sequenced (450-650 bp), and the same 299 bp region was obtained from other colonies from published data. Thirty-two sequences from Peninsula Valdès, Argentina, and 28 from South Georgia were from Hoelzel *et al.* (1993a), six sequences from Heard Island (HD) and five from Macquarie Island (MQ) were from Slade *et al.* (1998).

II.5 Genetic analysis

II.5.1 Paternity analysis

II.5.1.1 Characterisation of microsatellite loci

The pool of adult genotype data from Sea Lion Island was investigated and characterised. The level of polymorphism was estimated as the number of alleles per locus and the observed heterozygosity (H_O). Observed heterozygosity was tested for deviation from Hardy-Weinberg equilibrium (HWE) expectations for each locus with the Fisher's exact test as implemented in GENEPOP 3.3 (Guo and Thompson 1992). Fisher's exact test was also used for the evaluation of possible linkage disequilibrium (genotypic disequilibrium) and to test for differences between male and female allele and genotype distributions. All the analyses were performed in GENEPOP 3.3 using the Markov chain method (demorization number, number of batches, iteration per batch set at 1000) and applying the correction for multiple comparisons (Brown and Russell 1996).

II.5.1.2 Microsatellite identity check

Since the SLI samples of this study were collected during two seasons and in often difficult field conditions, an identity check analysis was performed in CERVUS 2.0 (Marshall *et al.* 1998) to find matching genotypes in the microsatellite genotype data (i.e. individuals sampled in both years but differently identified). Any duplicate was subsequently excluded from the genotype files prior to the paternity analysis.

II.5.1.3 Microsatellite null alleles

The null allele frequencies were calculated for each locus using CERVUS 2.0. A null allele is any allele that cannot be detected by the assay used to genotype individuals at a particular locus. With microsatellite loci, a null allele most often occurs because of mutations in one or both primer binding sites, sufficient to prevent effective amplification of the microsatellite allele. A locus with a null allele at high frequency usually shows a characteristic pattern of repeated homozygote-homozygote mismatches in known parent-offspring relationships. Mismatches of this type are identified in CERVUS 2.0 during parentage analysis. During the analysis CERVUS 2.0 treats mismatches generated by null alleles as if they were typing errors. Loci with high null allele frequencies (0.05 or more) should be excluded from parentage analysis, while those with lower null allele frequencies should not cause any problems (Marshall *et al.* 1998).

II.5.1.4 Paternity inference

In classical paternity inference, for each offspring as many as candidate males possible are excluded from paternity using the available genetic data. At the end of the procedure, if only one male is not excluded, the paternity is assigned to that male. However, this method alone offers no help when there are either multiple males who cannot be excluded as the father of a given offspring, or unsampled males. Paternity was therefore assigned following a likelihood-based approach using the program CERVUS 2.0 (Marshall *et al.* 1998). The program calculates the likelihood of each male being the true father of each pup, relative to the likelihood of it not being the true father (see Appendix A for a detailed description of the program).

CERVUS 2.0 was chosen over other available methods such as Goodnight & Queller's (1999) KINSHIP program or NEWPAT (Worthington Wilmer *et al.* 1999), for several reasons. First, because of its flexibility in assigning error rate, proportion of possible fathers sampled and proportion of loci used for genotyping. Second, because of the Δ statistic it uses to determine the most likely paternal candidate. This statistic allows defining a level of confidence for each paternity assigned (or not assigned). Third, because it has been reported to be more robust in discriminating between close relatives as possible fathers. Related potential parents can confound parentage assignment (Thompson & Meagher 1987). CERVUS 2.0, however, is robust to the confounding effects of most types of relatives, with only small over-estimations of confidence in most scenarios. The exception is when full-sibs of the offspring are also considered as candidate parents (Marshall *et al.* 1998) and this possibility was excluded in the analysis. Finally, it was chosen because of the options it gives in organising the 'offspring file'. It gives the possibility of choosing a different set of candidate fathers for each offspring and this was very useful since the two loci from BETA were used for a preliminary simple exclusion.

Simulation of paternity

The simulation analysis of CERVUS emulates the steps of paternity inference using allele frequencies of the screened loci. Assuming Hardy-Weinberg equilibrium, a maternal and a paternal genotype are generated from the allele frequencies observed, and an offspring is derived by Mendelian sampling of the parental alleles. Genotypes are also generated for a number of unrelated candidate males. The genotypic data for all individuals are then altered to reflect the existence of unsampled males, missing loci and incorrectly typed loci. Then, each candidate male is considered in turn as the potential father, and LOD score are calculated for all males for whom the genetic data exist. The most-likely and the second-most-likely males are identified and the Δ score calculated. Genetic data are generated and paternity tests carried out for a large number of simulated offspring in order to generate a distribution of Δ (10,000 tests).

The final stage of the simulation is to find critical values of Δ so that the significance of Δ values found in the paternity inference can be tested. For example, if a criterion is required for Δ which gives 95% confidence, the program identifies the value of Δ such that 95% of Δ scores exceeding this value are obtained by true fathers. When a male matching the 95% confidence criterion is assigned paternity of an offspring, the father-offspring relationship is described as a 95% confident paternity (Marshall *et al.* 1998). The simulation model needs some parameters in order to make simulated genetic data realistic:

a) number of candidate males: the number of males that are candidates for paternity of the offspring analysed. This includes unsampled parents and should be estimated from field observations;

b) proportion of candidate males sampled: the proportion of males for whom genotypic data are available;

c) proportion of loci typed: the proportion of loci typed averaged across all loci and individuals. This parameter allows for missing data;

d) error rate: the fraction of loci typed incorrectly (and null alleles), averaged across loci and individuals. In CERVUS, an error is defined as the replacement of the true genotype with a genotype selected at random under Hardy-Weinberg assumptions. Under this definition, an erroneous genotype will sometimes be the same as the true genotype. If mother-offspring pairs are known from field data, the error rate can be estimated from the frequency of mismatches between mothers and their offspring.

Paternity analysis on Sea Lion Island

In the analyses, each year was considered separately, with 115 mother-pup pairs and 78 males for 1996, and 77 mother-pup pairs and 62 males for 1997. Thirty-nine of the 78 males of the first year were also present in 1997, hence they were included in the analyses of both seasons.

Since the primer BETA amplified for two loci it was not possible to run its data in the program CERVUS 2.0. An initial screening on offspring and candidate males was hence carried out manually and with a program developed ad hoc in Hypercard™ software. Comparing the BETA alleles of each offspring with the BETA alleles of the candidate fathers, a list of putative fathers was selected for each offspring. The file containing the offspring with their individual list of candidates was then used as the 'offspring file' in CERVUS 2.0. Following this method, a male matching with the offspring in CERVUS 2.0 for the seven loci would also match in the BETA alleles previously checked.

Allele frequencies from males from both years (101 males) were used for the simulations. For each year, all behaviourally active males observed in the study area at some point during the breeding season were considered as candidate males. These also included some males of the JUV class even though, in practice, mating opportunities for males of this age are extremely infrequent.

In 1996 there were 81 males on land, of which 78 (96.2%) were sampled. In 1997 there were 68 of which 62 (91%) were sampled. The proportion of loci typed was 0.99 for both years and the error rate detected from mother-offspring genotypes was set equal to 0.4% and 0.2%, in 1996 and 1997 respectively. Paternity was assigned with 95% (strict) and 80% (relaxed) levels and 10,000 paternity simulations were generated. All these values were used in the simulations and details are in Table II.2.

Parameter	value in 1996	value in 1997
Number of candidate males	81	68
Proportion of candidate males sampled	0.962	0.912
Proportion of loci typed	0.999	0.999
Rate of typing error	0.004	0.002
Number of tests	10,000	10,000
Relaxed confidence level	80%	80%
Strict confidence level	95%	95%

Table II.2 The values of parameters used in simulation of paternity inference with the program CERVUS, for each year. The proportion of loci typed and the error rate are average values across the 7 loci screened

II.5.2 SLI population relatedness

Individual multilocus genotypes were used for estimating symmetrical pairwise genetic relatedness within the program KINSHIP 1.3.1 (Goodnight and Queller 1999). Levels of kinship were investigated within the SLI population and between the colonies of SLI and EI. Samples sizes in this analysis were 455 seals for SLI (101 adult males, 162 adult females and 192 pups) and 46 (16 females and 30 males) for EI.

KINSHIP 1.3.1 estimates Hamilton's relatedness coefficient (*R*) between two individuals, which measures the extent to which they have alleles that are identical by descent, using allele frequencies in the population and each individual genotype. The index weights each allele by its frequency in the population, so rare alleles are given relatively higher weight. It was calculated as:

$$R = \sum_{x}\sum_{k}\sum_{l}\frac{(P_{y} - P^{*})}{(P_{x} - P^{*})}$$

where x indexes individuals in the data set, k indexes loci, l indexes allelic position, P^* is the frequency of each allele in the population (excluding compared individuals – see below for 'unbiased calculations') and P_x and P_y are the frequency of each allele in compared individuals (Queller and Goodnight 1989). The coefficient ranges from −1 to +1. A positive R value indicates that two individuals share more alleles that are identical by descent than expected by change, while a negative R value indicates that two individuals shared fewer such alleles that expected by change. When either of the two individuals possesses uncommon alleles a negative R value is expected for a pair (de Ruiter and Geffen 1998). In a sample representing a population in Hardy-Weinberg equilibrium the relatedness coefficients should average 0.5 for parents and offspring or full-siblings (first-degree relatives), 0.25 for half-siblings and zero for randomly related individuals (Queller and Goodnight 1989).

To be completely unbiased when performing relatedness calculations, the R values should be calculated using allele frequencies estimated excluding the individuals whose relatedness is being calculated (Blouin *et al.* 1996). This is because individuals that are thought to be relatives of the individuals under consideration will be expected to have allele frequencies closer to those related individuals than to the true population mean. Their inclusion in the data set will then bias its measure of population frequencies in that direction (Queller and Goodnight 1989). To remove this kind of bias, the allele frequencies obtained from the EI population were used for the calculation of relatedness in the SLI population.

To ensure that the loci gave values of R close to those expected, relatedness coefficients were calculated from pairs of known relationships. The R values were obtained from 186 father-offspring pairs, 192 mother-offspring pairs, 30 half-sibling pairs sharing the same mother, and 1647 half-sibling pairs sharing the same father. Comparisons of individuals thought to be unrelated consisted of individuals from EI compared with adult seals from SLI. In this case, allele frequencies from SLI individuals showing R values smaller than 0.4 were used for the calculation.

Estimates of pairwise and mean relatedness were determined between females, males, females and males of SLI. Pup seals were excluded from this part of the analysis. The level of relatedness between different sets of individuals was compared using a Mann-Withney test, with Montecarlo method (20000 resamplings). The tests were carried out in the programs StatView 5.0 (SAS Institute) and StatXact Turbo 4.0 (Cytel Software Corporation). In case of multiple comparisons, sequential correction was applied (Holm 1977).

II.5.3 Population genetic structure

II.5.3.1 Microsatellite population analysis

For the microsatellite analysis, individuals from SLI (263) and EI (46) were screened for 9 microsatellites loci (the primer BETA amplified for two loci). Data from 95 individuals from the Peninsula Valdés (PV) and 46 individuals from South Georgia (SG) were from Hoelzel *et al.* (2000) and analysed at 5 of the seven loci (Hg6.3, Hg8.10, Hg8.9, M11a, M2b). Frequencies at the loci BETA from SLI and EI were compared with published data for HD, MQ (Slade *et al.* 1998), and SG (Hoelzel *et al.* 2001).

Population polymorphism

Genetic polymorphism of each population was inferred using the program GENETIX v.4.01 (Belkhir *et al.* 2001). It was measured as mean number of alleles per locus, observed heterozygosity (H_O) and heterozygosity expected at each locus from Hardy-Weinberg assumptions (H_E). The unbiased expected heterozygosity at each locus in every population was estimated as:

$$H_E = \frac{2n(1-\sum p_i^2)}{(2n-1)}$$

where p_i is the frequency of each of the alleles at a locus and n is the number of individual sampled (Nei 1987; p. 178, eqn 84).

Since the observed number of alleles in a sample is dependent on sample size, allelic richness per locus and per population (R_S) was estimated as implemented in the program Fstat 2.9.3 (Goudet 1995, 2001). Allelic richness is a measure of the number of alleles independent of sample size and hence allows comparing this parameter between different sample sizes. The principle is to estimate the expected number of alleles in a sub-sample of *2n* genes, given that *2N* genes have been sampled ($N \geq n$). In Fstat 2.9.3, n is fixed as the smallest number of individuals typed for a locus in a sample and *Rs* is calculated as:

$$R_s = \sum_{i=1}^{n_i} \left[1 - \frac{\binom{2N - N_i}{2n}}{\binom{2N}{2n}} \right]$$

where N_i is the number of alleles of type i among the *2N* genes. Each term under the sum corresponds to the probability of sampling allele i at least once in a sample of size *2n*. If allele i is so common that it will be certainly sampled, the ratio is undefined but the probability of sampling the allele is set to 1.

To assess whether there was random mating within the populations (i.e. if the different populations were representative of separated and independent breeding colonies), the Wright's inbreeding coefficient, F_{IS}, was calculated. It measures the correlation of genes within individuals belonging to the same subpopulations and it was also calculated with the program Fstat 2.9.3. Weir and Cockerham's (1984) estimator (f) of F_{IS} was calculated:

$$f = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2}$$

where σ^2_b is the between individual variance component and σ^2_w the within individual component.

Deviations from Hardy-Weinberg equilibrium (null hypothesis: random union of gametes) were tested for each population using an exact test, available in GENEPOP 3.3. The program uses a Markov chain method to estimates values of Fisher's exact test (Raymond and Rousset 1995). Demorization number, number of batches, iteration per batch were all set at 1000 and correction for multiple tests was applied (Brown and Russell 1996). Linkage disequilibrium (null hypothesis: genotypes at one locus are independent from genotypes at the other locus) was tested for each pair of loci in the same program.

Population differentiation

Population genetic differentiation was tested using three different approaches. First, differences in allelic and genotypic distributions (null hypothesis: the allelic (or genotypic) distribution is identical across populations) for all pairs of populations were evaluated in GENEPOP3.3, using Fisher's exact test.

Second, estimates of Wright's fixation index, F_{ST}, were computed using the program Fstat 2.9.3 (Goudet 2001), which estimates confidence intervals bootstrapping over loci. The statistic is based on the variance in allele frequencies and assumes an infinite mutation model (IAM). It describes the proportion of variation in subpopulations relative to total variance:

$$F_{ST} = \frac{S_t - S_w}{S_t}$$

where S_t and S_w are proportional to the total and within population variances, respectively. This value relies on the assumption that all populations have descended from a common ancestor, that they are maintained under the same conditions, and that gene frequencies are at equilibrium (Balloux and Lugon-Moulin 2002). Weir and Cockerham's estimator (θ) of F_{ST} was calculated by the program as:

$$\theta = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_b^2 + \sigma_w^2}$$

where σ^2_a is the among sample variance component, σ^2_b is the between individual variance component and σ^2_w the within individual component.

Third, the unbiased R_{ST} statistic was computed using the program RSTCALC (Goodman 1997b). It is analogous to F_{ST} but it is based on the variance in repeat number between alleles and is assumes the stepwise mutation model (SMM) (Slatkin 1995):

$$R_{ST} = \frac{S_t - S_w}{S_t}$$

where S_t is twice the estimated variance in allele size across populations and S_w is twice the average of the estimated variance in allele size within each population. This measure, however, assumes populations of equal sample size and that all loci have equivalent variance. RSTCALC deals with both sources of bias and calculates an unbiased estimate, Rho_{ST}. This value represents the fraction of the total variance of allele sizes that is due to genetic differences between populations. RSTCALC calculates the statistical significance of Rho_{ST} by permutation tests, and uses bootstrapping to provide 95% confidence intervals. The number of interactions was set at 1000. Both F_{ST} and R_{ST} estimators range from 0, which signifies no differentiation, to 1, which indicates complete differentiation between populations.

Genetic distance between populations was estimated using Nei's unbiased genetic distance, D_A (Nei 1983), and $(\delta\mu)^2$ (Goldstein *et al.* 1995). D_A was calculated with the program GeneDist (available at: http://www.biology.ualberta.ca/jbrzusto/GeneDist.html):

$$D_A = 1 - \frac{1}{r}\sum_{j}^{r}\sum_{i}^{m_j}\sqrt{x_{ij}y_{ij}}$$

where x_{ij} and y_{ij} are the frequencies of the *i*th allele at the *j*th locus in population X and Y respectively, m_j is the number of alleles at the *j*th locus and r is the number of loci examined. D_A is independent of the mutational methods and computer simulation with the IAM has shown that this distance is more efficient than other Nei's distances like D_S or D_m (Takezaki and Nei 1996).

The distance $(\delta\mu)^2$ was proposed by Goldstein *et al.* (1995). It was created for microsatellite loci and incorporates features of the stepwise mutation model. It was calculated with the RSTCALC program and it is given by:

$$(\delta\mu)^2 = (\mu A - \mu B)^2$$

where μA and μB are the mean allele sizes in populations A and B respectively. The program calculates them by first finding the average allele size at each locus in each population. The squared difference in mean allele size is then averaged over loci. If reproductive isolation, mutational drift equilibrium and stepwise mutation are verified, then $(\delta\mu)^2$ is a linear function of the separation time between populations (Goldstein *et al.* 1995).

Population assignment

To study the likelihood of finding one of the observed genotypes in each of the populations (i.e. to determine how indicative an individual's genotype was of the population in which it was sampled) an assignment test was implemented in the program Doh (Paetkau *et al.* 1995; Waser and Strobeck 1998 – available at: http://biology.ualberta.ca/jbrzusto/Doh.html). The test calculates the likelihood of finding a given genotype in each population and assigns each individual to the population for which they had the highest likelihood. The calculation is based on the observed distributions of the alleles and assumes random mating and HWE (null hypothesis: populations are actually one well-mixed population in HWE). The null hypothesis was tested with 3000 randomisations. To avoid likelihood values of zero the correction suggested by Titterington *et al.* (1981) was used. The output of the program consists also of a matrix showing how often (i.e. in how many randomisation datasets) the cross assignment from row (nominal population) to column (assigned population) was at least as large as in the assignment test run without randomisation. This number divided by the number of randomisations gives an estimate of the probability of observing at least as many cross assignments if the appropriate null hypothesis is true.

II.5.3.2 mtDNA population analysis

For the mtDNA analysis, samples from SLI (30 males and 27 females) and EI (15 males and 15 females) were sequenced and analysed. Sequences from SG (24 sequences from 28 seals) and PV (3 sequences from 32 seals) were from Hoelzel *et al.* (2000) and were included in the analysis. Sequence from MQ (5 sequences from 5 seals) and HD (6 sequences from 6 seals) were from Slade *et al.* (1998) and they were used in only part of the analysis due to their small sample sizes.

Level of polymorphism

Level of polymorphism (π) was measured as nucleotide diversity (Nei 1987), assuming the Tamura-Nei (1993) model of sequence evolution and heterogeneity in the substitution rates across nucleotide sites following a gamma distribution with a value of $\alpha = 0.4$. The Tamura-Nei correction assumes higher rate of transitional substitutions than that of transversional substitutions and also allows for different rates between purine (A and G) transitions and pyrimidine (T and C) transitions. Nei's (1987) measure of gene diversity ($\hat{H}$) was also calculated. It is the equivalent of the expected heterozygosity for diploid data and it is defined as the probability that two randomly chosen haplotypes are different in the population:

$$\hat{H} = \frac{n}{n-1}\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

where n is the number of gene copies, k is the number of haplotypes and p_i is the sample frequency of the i-th haplotype. The calculations were run in the program ARLEQUIN 2.1 (Schneider *et al.* 2001).

A mismatch distribution test was run for each population. The test analyses the observed distribution of the nucleotide site differences between pairs of haplotypes and compares it with the expected distribution. This is usually multimodal in samples drawn from populations at demographic equilibrium and unimodal in populations having passed through a recent demographic expansion (Rogers and Harpending 1992). The test employed in ARLEQUIN 2.1 uses a bootstrap approach that was set to 1000 replicates. The program computes the *raggedness index* (r) of the observed distribution, which takes larger values for multimodal distributions commonly found in stationary populations than for unimodal and smother distributions typical of expanding populations.

Each population was analysed with Tajima's test of selective neutrality, which is based on the infinite-site model without recombination (Tajima 1989). Values of Tajima's D were tested for the hypothesis of selective neutrality and equilibrium in DNASP 3.5.3 (Rozas and Rozas 1997) by generating 10000 random samples. It is important to note that significant D values can also be due to factors other than selective effects, like population expansion, bottleneck or heterogeneity of mutation rates. A change in population size can result in deviations from the neutral patterns of nucleotide variation expected at equilibrium. In a population of constant size, variation at a neutrally evolving locus is expected to a have a D value of approximately zero. Following a reduction in population size, rare frequency mutations are lost more readily than are common mutations and transient positive D values are expected. On the contrary, following an increase in population size there is a temporary excess of new mutations segregating at rare frequencies, and negative D value are expected (Fay and Wu 1999).

Fu's test (Fu 1997) of selective neutrality was also run on each sample in the same program, generating 10000 random samples. As for the Tajima's test, it is based in the infinite-site model without recombination. It evaluates the probability of observing a random neutral sample with a number of alleles similar or smaller than the observed value. The statistic F_S is very sensitive to population demographic expansion, which generally leads to large negative F_S values (Schneider *et al.* 2001).

Genetic differentiation between populations

Genetic differentiation between populations was quantified and tested with the F_{ST} statistics (estimator ϕ_{ST}). Population pairwise ϕ_{ST} were calculated in ARLEQUIN 2.1 on both sequence data (distance method: Tamura-Nei, $\alpha = 0.5$) and sequence haplotype frequencies (Weir 1990). Permutations for significance was set to 10000 and P-value represented the proportion of permutations showing a ϕ_{ST} greater or equal to the observed one.

Genetic distances between populations were calculated using the program DNASP 3.5.3. Uncorrected nucleotide divergence values (D_{xy} and D_a) were calculated by the program following Nei (1987; equations 10.20 and 10.21). D_{xy} represents the average number of nucleotide substitutions per site between populations, and D_a the number of net nucleotide substitutions per site between populations (i.e. gross divergence minus within-population diversity). The total number of shared mutations and the number of fixed differences between populations (i.e. nucleotide sites at which all of the sequences in one population are different from all of the sequences in the second population) were also calculated with the same program.

Phylogenetic relationships among the control region sequences were analysing following different approaches. First, a maximum parsimony (MP) analysis was run with PAUP 4.0b10 (Swofford 1998), using a northern elephant seal sequence as outgroup. A majority-rule consensus tree was constructed from 1000 bootstrap replications and a 50% criterion for the retention of nodes was applied. Some of the mutations between sequences were transversions, therefore the transition/transversion ratio was calculated and the value (6.27) used in the phylogeny construction. Second, with the same computer program, a neighbour-joining (NJ) tree was also constructed. It was constructed from 1000 bootstrap replications and the distance matrix was based on the Tamura-Nei model and a gamma distribution with $\alpha = 0.5$. Finally, the pattern of sequence evolution was illustrated with a network, in which sequences are the nodes of a network rather than the terminal tips of a tree. A median joining network was constructed in the program NETWORK 31000A (available at: www.fluxus-engineering.com) following Bandelt *et al.* (1999). An approach using networks has some advantages: the network can predict haplotypes, it can show which sites mutated frequently, where the consensus sequence is and it can specify mutational events (Bandelt *et al.* 1995). In the network graphical display, each node represents either an observed haplotype or a hypothetical intermediate haplotype; the area of the circle representing each haplotype is proportional to the number of individuals with that haplotype; the length of the links is proportional to the number of mutations

III Male reproductive success: behavioural estimates and paternity

III.1 INTRODUCTION

III.1.1 Behaviour and genetics

Studies in behavioural ecology are traditionally based on measures of individual reproductive success to define the mating system of natural populations (Clutton-Brock 1989) or on phenotypic and behavioural traits correlated to reproductive success and under selective pressure (Dewsbury 1982). Analyses involving molecular markers, however, have frequently demonstrated that mating behaviour does not always reflect parentage and may indicate alternative mating tactics.

Previous studies on polygynous mammals have revealed different results. In a red deer population (*Cervus elaphus*), behavioural data reflected parentage, but slightly underestimated the variance in seasonal and life-time male reproductive success (Pemberton *et al.* 1992). In Soay sheep (*Ovis aries*), census-based observations were less good predictors of paternity and the level of polygyny was lower than expected (Coltman *et al.* 1999a). In Pinnipeds, studies on grey seals (Worthington *et al.* 1999; Ambs *et al.* 1999) and Antarctic fur seals (Gemmell *et al.* 2001) showed notable discrepancy between genetics and behaviour. In both species, behaviour-based measures over estimated male mating success and hence the level of polygyny in the study populations. These investigations show that the effectiveness of behavioural methods in assessing paternity varies with species and population. Therefore the relationship between the behavioural and genetic data should be carefully assessed in each study.

III.1.1.1 Mirounga

Behavioural studies on *Mirounga* predict a high variance in both seasonal (Le Boeuf 1974; McCann 1981) and lifetime male reproductive success (Le Boeuf and Reiter 1988). In a harem, the holder is expected to achieve the majority of matings, and the percentage he achieves is predicted to be greater in smaller harems, with lower competition pressure, than in larger harems, that are usually frequented by many peripheral males. In a study where behavioural and genetic success of harem holders was compared for the two species of *Mirounga* (Hoelzel *et al.* 1999), behavioural estimates were most of the time consistent with genetics for harems of southern elephant seals (SES), while they tended to over estimate genetic paternity in harems of northern elephant seals (NES). SES holders from the Argentinean population were more successful than NES. In harems from 30 to 119 females SES holders achieved a mean of 58% paternities (range 50-70%; n paternities analysed for each harems = 2-19). In contrast, NES holders achieved a mean of 38.9% paternities per harem (range 0-100%; harem size = 9-224; n paternities analysed for each harem = 1-18). Variance in NES holder reproductive success was lower than expected and reproductive success of peripheral males greater.

For the SLI population, evidence from behavioural observations and previous research (Fabiani 1996; unpublished data) support the hypothesis that the most successful male mating tactic is to get the control of a harem. However, harems often have peripheral males that always attempt to gain access to oestrous females by adopting ‘sneaky’ tactics. It is thus possible that their reproductive success will be underestimated by behavioural observations. If this was the case, the variance in male reproductive success and the degree of polygyny will be reduced and hence the pressure of sexual selection less pronounced.

Another important factor in investigating reproductive success is the local demography of the population. In natural populations, different local demography may result in a spatial variation of sex ratio and in consequently variation in distribution of male mating success. Significant differences were found between the elephant seal populations of Delgada (DEL, Peninsula Valdés) and Sea Lion Island (SLI) when the local demography of the two populations was taken into account (Galimberti *et al.* 2002a). When male mating success was analysed at harem level, variance in male mating success and opportunity of selection (I) varied both between colonies and among harems. I calculated among harems had a mean of 4.9 ± 2.3 at SLI (n harems = 12, over four breeding seasons) and 7.8 ± 3.9 at DEL (n harems = 15, over 2 breeding seasons), and the difference between the two populations was highly significant ($P = 0.0281$ – Details in Galimberti *et al.* 2002a).

In this study different harems from different breeding seasons were analysed, in order to evaluate the effectiveness of behavioural measures in various contexts and to develop a more accurate picture of the mating system in the population. Each harem was treated as an autonomous breeding unit, with its females, holder and peripheral males, when present. This was possible because usually females do not move to other harems after giving birth and holders rarely leave their female groups. This allowed the analysis of mating and paternity distributions for each harem, and to relate the distributions to the harem's reproductive context. Each harem has a different social composition, characterised by the presence of peripheral males, the eventual change of the holder, by the number of breeding females and by the possible splitting or merging of the unit. The identity of the holder can change during the breeding season if a dominant male chases him and successfully take over the female control. Similarly small and close harems can increase in number and merge into a single big unit, or a large group of females can split into two groups, each one controlled by a single male.

Variation in male mating success and paternity is shown as standardised variance (i.e. variance divided by the square of the mean, as in Wade and Arnold 1980). This measure, opportunity of selection (*I*), represents the upper limit of the potential action of phenotypic selection in each analysed system (group, population, species). It has been widely used for comparing mating systems both within and among populations and species (Clutton-Brock 1988). Opportunity of selection or a similar measure (i.e. variance divided by the mean, see Boness *et al.* 1993) have also been frequently calculated in studies of pinniped mating systems (Le Boeuf and Reiter 1988, Coltmann *et al.* 1998, Twiss *et al.* 1998) and when levels of polygyny were investigated in other species (Packer *et al.* 1988; Cheney *et al.* 1988; Altmann *et al.* 1988). A truly monogamous mating system will show a variance of zero, while a mating system with high level of polygyny will have a much larger value.

III.1.2 Reproductive context on SLI

On SLI, females begin to haul out during the second week of September. Each year, the daily variation in the number of females on land follows a bell-shaped curve (Galimberti and Sanvito 2001) with a maximum in the same (or almost the same) day in different seasons (20th of October in both 1996 and 1997, 19th in the 1998). Almost all the females go back to sea by the third week of November and matings are centred over a short time, with about 90% of the females coming into oestrus in a 3-week period (Fig III.1).

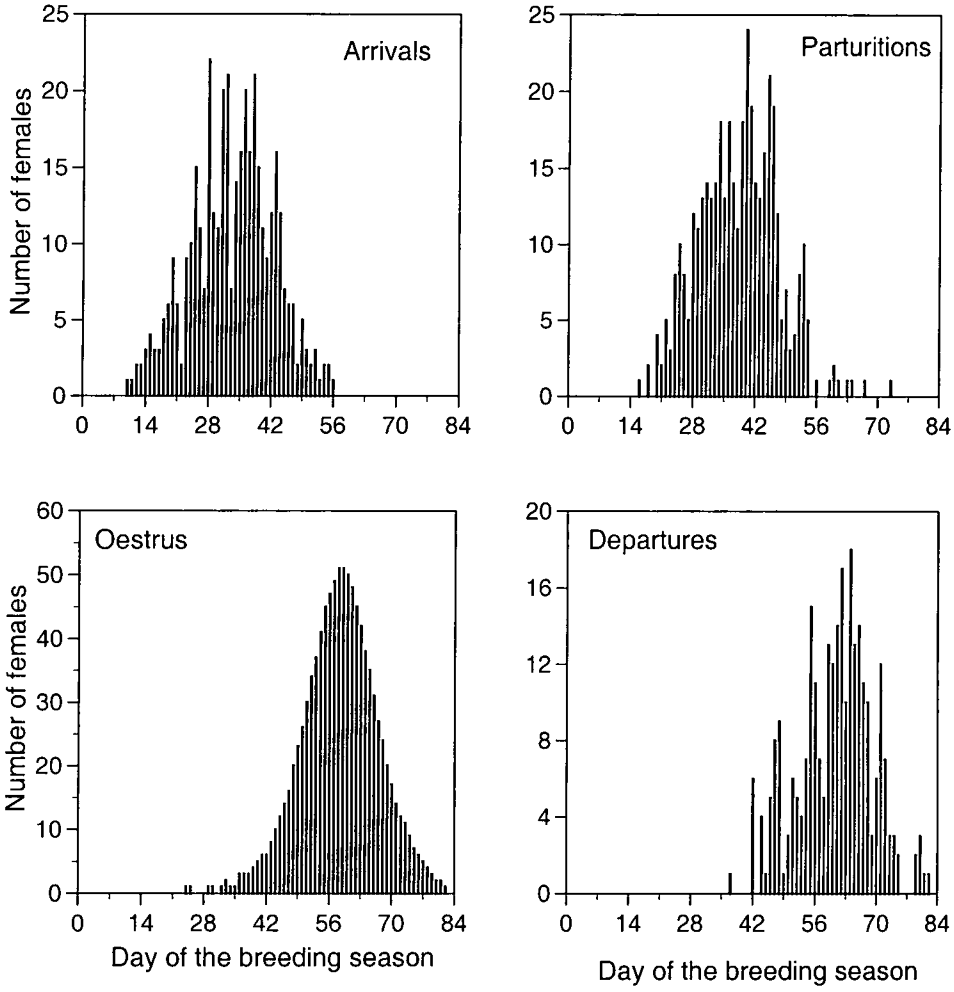

Fig III.1 Distribution of days of occurrence of female breeding events on SLI. The data are from 1996 and sample sizes are as follows: Arrivals, n = 394 females; Parturitions, n = 406; Oestrus, n = 404; Departures, n = 275 (from Galimberti and Boitani 1999)

Since male reproductive success is limited by the availability in time and space of the oestrus females, this high female gregariousness and the oestrus synchronisation provide the potential context for a high level of polygyny and action of sexual selection (Emlen and Oring 1977). With oestrus females clumped in harems, the possibility arises for a reduced number of males to monopolise them, and for variance in male reproductive success to be consequently large.

During the study breeding seasons, the seasonal breeding sex ratio (*BSR* = number of breeding females/number of breeding males) followed the distribution of females on land and seasonal means were 9.1 in 1996 and 10.1 in 1997. In a polygynous species with a harem defence mating system a better measurement of the actual sex ratio of breeding individuals may be to consider only the harem holders, since they should have almost unique breeding access to the females. On the island, the mean seasonal ratio of breeding females to harem holders was 46 and 47, respectively. This value is very similar to the range of 41-53 reported for the colony of Delgada in Peninsula Valdès (Galimberti *et al.* 2000a). It is intermediate between the value for the entire Peninsula Valdès by Campagna *et al.* (1993) (11 females/harem holder) and those reported for subantarctic populations, e.g. 74.2 females per harem holder for South Georgia and 277 for Macquarie (McCann 1980). However, data on sex ratio should be interpreted with caution because there may also be a great variation in sex ratio within population, i.e. among different breeding seasons. A more reliable measure that indicates the variation of male mating opportunity during the breeding season is the operational sex ratio (*OSR*), which is the ratio between fertilisable females and reproductive males at any given time (Emlen and Oring 1977). Due to the female breeding synchronisation in southern elephant seals, the number of females on land is not a good indicator of the number of oestrus females available for mating. In this context, this measure might have a more important role than the simple *BSR* in influencing male mating behaviour during the season. Very few females were estimated being in oestrus every day (for 1996: daily mean of oestrus females = 12 ± 16, med = 3, MAD = 3, range 0-51) and contrary to the *BSR*, the *OSR* distribution did not follow the number of females on land (Fig III.2).

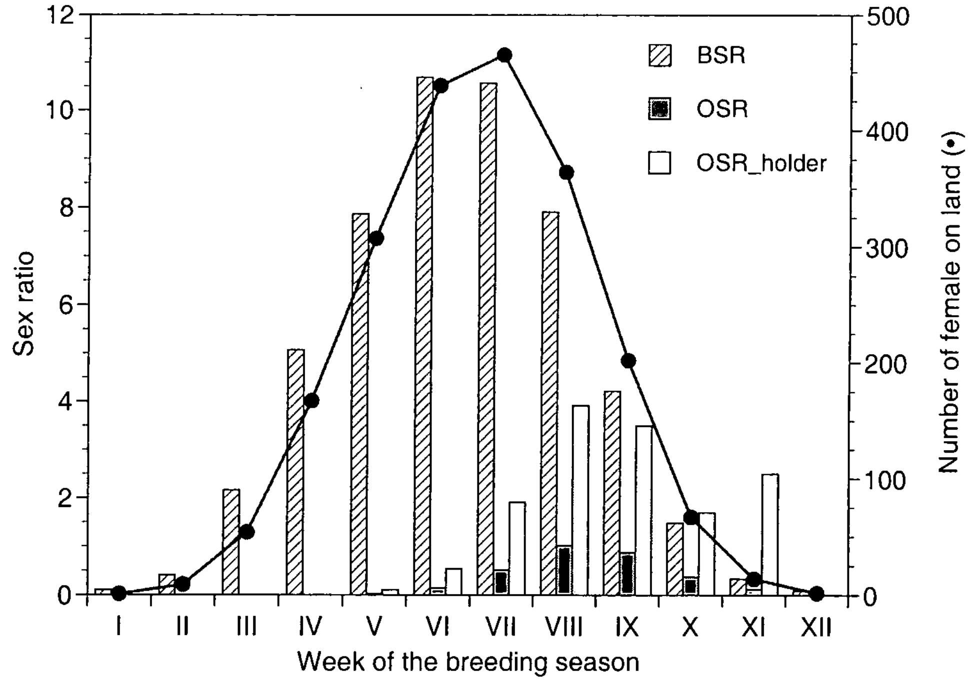

Fig III. 2 Weekly variation of sex ratios with females on land in 1996. The breeding sex ratio (*BSR*) follows the distribution of females in the colony, while the operational ratios (*OSR*, and *OSR_holder*) follow different distributions, with their maximum towards the second half and end of the season

III.1.3 Aims

In this chapter, the degree to which behavioural observations reflected genetic mating success was tested. In particular, behavioural and genetic data were used to:

a) evaluate if the estimates of male breeding performance, described by the distributions of a female control index, the observed copulations and a fertilisation estimate, were useful predictors of the distribution of paternity, both in different harem contexts and in the population;

b) determine the distribution of paternity, quantify its variance and test if it was skewed towards a small number of males and representative of a highly polygynous mating system;

c) test if the paternity assigned to each pup using behavioural criteria was a good predictor of genetic paternity.

III.2 RESULTS

III.2.1 Level and distribution of genetic diversity

III.2.2.1 Identity checking

The identity check analysis was performed in CERVUS 2.0 to test eventual matching between genotypes (i.e. same individual sampled more than once). Three genotype matches were found for three males that were present during both breeding seasons and known with different identities. The three males were present in 1996, when they were identified and tagged. They returned in 1997 with signs of at least two lost tags, but the lack of natural marks made their identification impossible. With the genotype checking, the three males of 1996 were identified as the same three males of 1997. The duplicates were excluded from the analysis.

III.2.1.2 Microsatellite characterisation

No significant differences in allele and genotype frequencies were detected between males and females in the population. The adult genotypes were therefore pooled and analysed together.

Each locus was tested for Hardy-Weinberg equilibrium (null hypothesis of random union of gametes), using the Fisher exact test as implemented in GENEPOP 3.3. None of the P-values were significant and none of the loci could be considered as deviating from Hardy-Weinberg expectations. The result was confirmed by testing the samples for heterozygote deficiency (score test – U test) in the same program.

Each pair of loci was tested for linkage disequilibrium in GENEPOP 3.3. The test assumes the null hypothesis of independence between genotypes at one locus and genotypes at the other locus. The seven loci were found to be independent from each other. The null allele frequencies were calculated in CERVUS 2.0. The null allele frequencies ranged from -0.007 to 0.042. Being lower than 0.05, they were considered as not interfering with the analysis (Marshall *et al.* 1998). Details of the microsatellites used are given in Tables III.1 and III.2.

Locus	n alleles	H_e	H_O	Null Allele Frequencies
Hg4.2	4	0.57	0.58	-0.007
Hg6.3	7	0.62	0.57	0.042
Hg8.10	6	0.76	0.78	-0.020
Hg8.9	9	0.70	0.68	0.012
M11a	7	0.77	0.78	0.007
M2b	10	0.73	0.73	-0.011
Pv9	5	0.45	0.46	-0.018

Table III.1 Polymorphism characteristics, number of alleles, expected (H_E) and observed (H_O) heterozygosities, estimated frequency of null alleles (Marshall *et al.* 1998) for the microsatellites used (adult genotypes)

	Beta Alleles (size in bp)														
	269	*274*	*279*	*284*	*289*	*294*	*299*	*304*	*309*	*314*	*319*	*324*	*329*	*334*	*339*
Count	40	13	51	49	230	331	108	18	2	62	34	17	49	44	4
Percent	3.8	1.24	4.85	4.66	21.86	31.46	10.27	1.71	0.19	5.89	3.23	1.62	4.66	4.18	0.38

Table III.2 Adult allele frequencies and percentages for the primer Beta

III.2.2 Mating distribution and breeding performance

A total of 846 copulations were recorded in the study area during observation periods in 1996 and 1997 (September-November). Only 28.2% of the males in the colony were observed mating at least once in the two breeding seasons (n males = 149). The copulation distributions (Fig III.3) did not differ between the two years (Mann-Whitney: $U = 2801.0$, $P = 0.821$, $n_{1996} = 81$, $n_{1997} = 68$) and had a mean of 5.65 ± 15.97 for all males (*med* = 0, *MAD* = 0) and of 20.05 ± 25.0 for males that copulated (n = 42, *med* = 7, *MAD* = 6). The two distributions gave an opportunity of selection (*I*) of 7.99 and 1.55, respectively. Among males that copulated, 54.8% achieved less than 10 copulations, 35.7% more than 20.

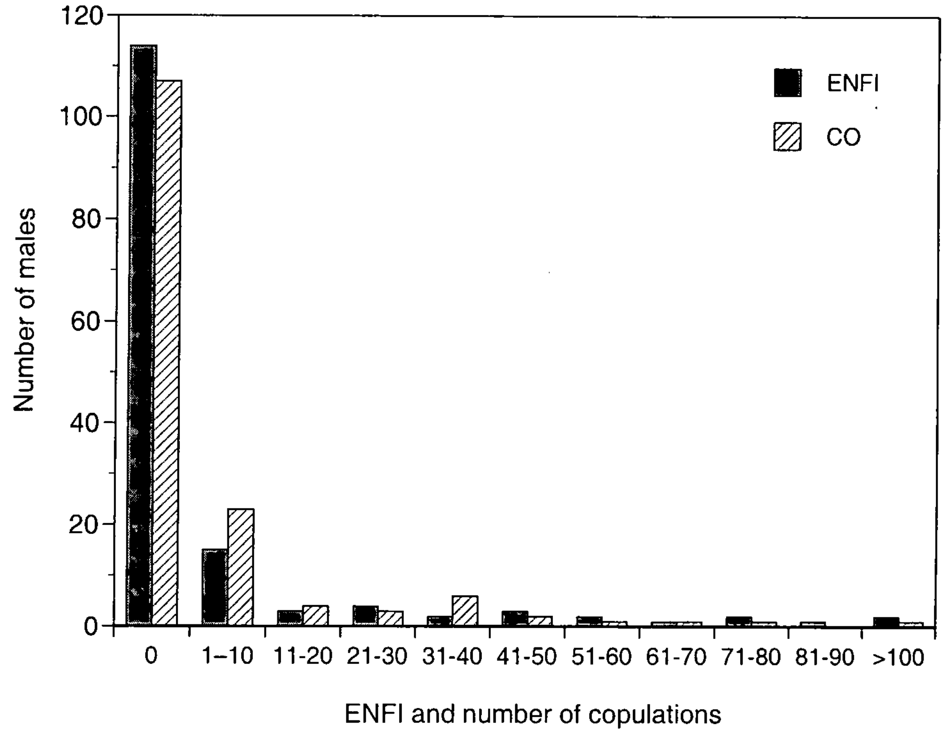

Fig III.3 Distributions of the fertilisation index (*ENFI*) and of copulations (n° CO) among males in the population. The two years are shown together, for a total of 149 males (81 in 1996 and 68 in 1997)

In the two years, only one male had more than 100 copulations recorded, with 108 copulations in 1996. This same male also achieved the highest number of matings in the following season (66 copulations in 1997).

The distributions of *MS100* (mating index, calculated as the number of copulations recorded for each male in 100 hours of observation) and *ENFI* (fertilisation index, from the proportion of copulations achieved by each male in each harem and the females breeding in the harem – see section II.3.1) were very skewed across each breeding season ($g1$ = 2.95 and 3.56). They both had a median value of 0 and the variation among individuals was very large (range 0-59 and 0-125, CV = 2.40 and 2.89). The opportunity for selection resulting from the two distributions was 5.91 for the mating index (mean = 3.84 ± 9.22) and 8.14 for the fertilization index (mean = 7.11 ± 20.53). The distribution of *FFD* (female control index, sum of the females controlled by each male throughout the season) was also very skewed ($g1$ = 3.62) and ranged from 0 to 3541 (mean 187.29 ± 552.24). The data from daily censuses showed that 40.6% of the males were never seen associated with a female during the whole breeding season (Fig III.4).

The period a male stayed on land for reproduction (here defined as *tenure*) largely varied (range 5-83 days, n males = 149). There was a positive correlation between *FFD* and the days of *tenure*, considering both all males and males that held females (*rho* = 0.76 and 0.71, $P < 0.0001$). Males that held females during the breeding season stayed a significantly longer period on land compared to males that did not hold females (Mann-Whitney: U = 5368, $P > 0.0001$; n males that held females = 88, n males that did not hold females = 61) for a median value of 58 (*MAD* = 15, range 14-88) versus 27 days (*MAD* = 8, range 5-69). Sixty two percent of the males who had zero associated females recorded stayed on land for less than 30 days.

III.2.2.1 Holders and non-holders

A significant difference in breeding performance was expected between males that achieved the control of a harem and males that did not. In the analysis of the three indices, there was a highly significant difference when principal and non-principal males were compared (Mann-Whitney: $U > 3100$ and $P < 0.0001$ for each distribution; n *principal* males = 27, n non-*principal* males = 122).

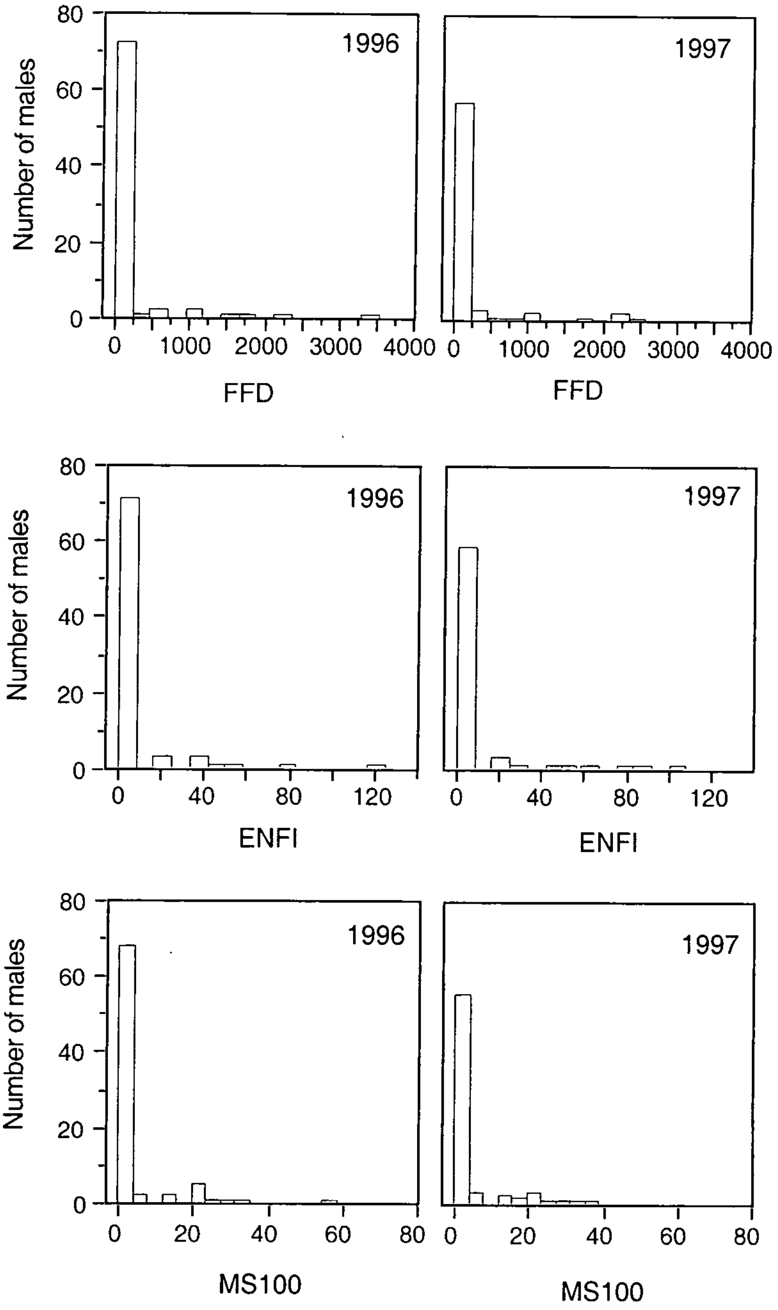

Fig III.4 Distribution of the breeding indices each year. *FFD* = female control index; *ENFI* = fertilisation index and *MS100* = mating index

Variation in the mating performance was smaller, but still present, when only the seasonal harem holders were considered. Five out of 20 harem holders (25%) achieved twenty or less copulations, and the *ENFI* distribution had a mean of 49.9 ± 32.1 (*med* = 41, *MAD* = 20, *CV* = 0.64). The distributions of *MS100* and *FFD* were similarly skewed and showed analogous variation (*CV* = 0.47 and 0.72) (Fig III.5). There was no significant difference in any of the mating performance indices between harem holders in the two years.

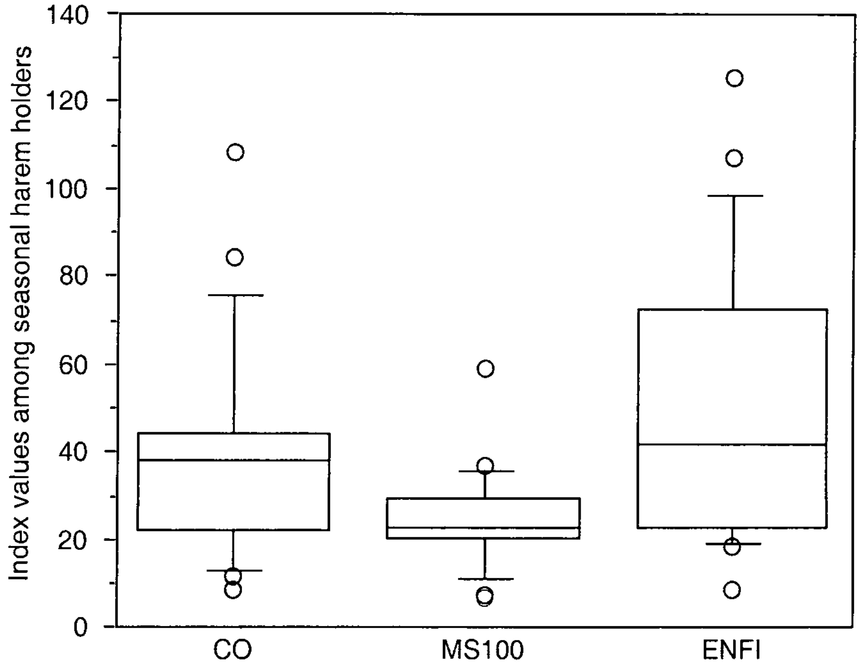

Fig III.5 Distribution of copulations (CO), fertilisation (*ENFI*) and mating success (*MS100*) indices among principal males (i.e. males that gained the control of a harem for at least 24 hrs; n = 27 males)

III.2.2.2 Secondary and tertiary males

Among males that did not gain the control of any harem, matings were not evenly distributed. Males associated with a breeding unit (secondary males) had higher success than males never observed associated with a harem (tertiary males) (for *MS100*: Mann-Whitney: *U* = 2226.0, *P* = 0.044; n secondary males = 68, n tertiary males = 54). Of the 20% of secondary males that mated at least once, two males had seven and one had nine copulations recorded. Among the tertiary males, only one male achieved four copulations. His copulations were all recorded during the same observation period, when the harem holder was absent for a short period of time. The difference between associated and non-associated males was larger when the female control index (*FFD*) was considered (Mann-Whitney: $U = 2862.5$, $P < 0.0001$) but was not significant for *ENFI* (Mann-Whitney: $U = 2096.5$, $P = 0.179$). The *MS100* distributions had a mean of 0.69 (± 1.95) and 0.27 (± 2.00) for secondary and tertiary males respectively, while the equivalent *ENFI* values were 0.412 (± 1.14) and 0.056 (± 0.41).

III.2.3 Mating distribution in the harems

For a more detailed analysis of the relationships between behavioural and genetic data, seven harems belonging to only one zone of the study area were chosen (five for the first year and two for the second). The males associated with each harem were recorded during the daily censuses, and their mating performances were estimated from the data collected during the standard observation periods.

III.2.3.1 Socionomy of the harems

As for the whole population (Galimberti and Sanvito 2001), the daily variation in the number of females in a harem was best described by a Gaussian distribution; the peak of the distribution was defined as the harem size and represented almost 90% of all the females that bred in the harem (Fig III.6).

The size of the seven harems ranged from 18 to 91 females (mean = 47.71 ± 27.52) and the number of different associated males recorded during the whole season for each harem ranged from 0 to 13 (mean 8.00 ± 5.53). Considering both the harem holder and the males associated with the harem, the mean daily breeding sex ratio (*BSR* = number of breeding females/number of breeding males) ranged from 9.04 to 22.21, with a maximum of 74. The variation in the mean daily number of associated males was large both among ($CV = 1.84$) and within harems (*CV* range 0.97-3.18). Data from censuses also showed a significant difference in the daily number of males associated with the seven harems (Kruskal-Wallis: $KW = 84.98$, df = 6, $P < 0.0001$; 42-58 censuses on each harem). Details of the breeding units are given in Table III.3.

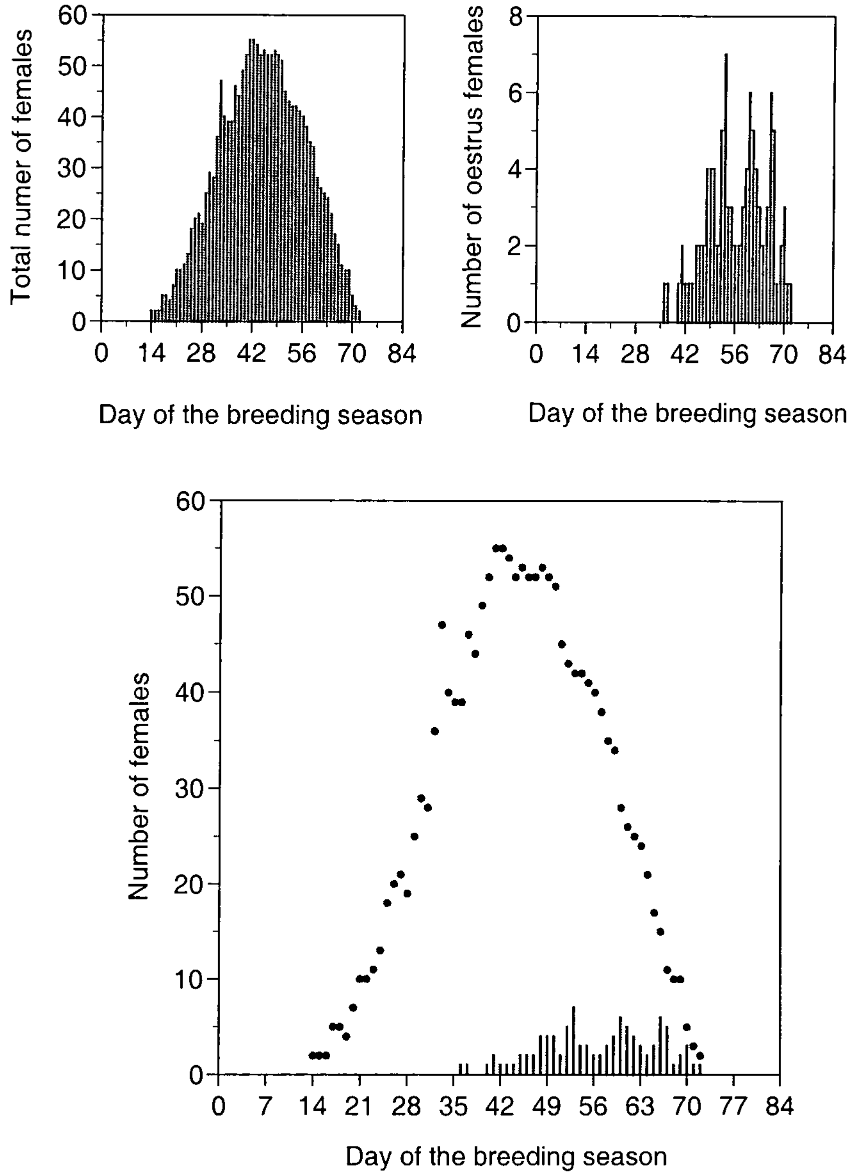

Fig III.6 Number of females in the harem SI196 each day of the breeding season (the harem formed on the 14th day of the season). Up left: total number of females in the harem; up right: number of oestrus females; below: total females (dots) and oestrus females (lines) together

Harem	Size	mean (± sd) daily assoc. males	max daily assoc. males	total assoc. males	*BSR* (± sd)
RUB96	18	0.29 (± 0.56)	2	11	9.04 (± 4.40)
SF96	35	0.122 (± 0.39)	2	2	17.25 (± 9.34)
SI196	55	0.66 (± 0.78)	2	13	18.02 (± 9.52)
SI296	75	1.25 (± 1.15)	4	13	16.88 (± 12.74)
SM96	20	0.156 (± 0.47)	2	5	10.86 (± 5.96)
SF97	40	0	0	0	20.94 (± 11.76)
SI297	91	0.95 (± 1.46)	5	12	22.21 (± 18.59)

Table III.3 Demographic and social details of the harems analysed: name and size of the harem; mean (± standard deviation) and maximum daily number of males associated with the harem; total of different males associated during the breeding season; mean breeding sex ratio (*BSR*). Peripheral and marginal males (not the harem holders) are considered as associated males

There was a weak but significant correlation between the number of males associated each day with the harem and the number of females each day in the harem (*rho* = 0.5, P_{10K} = 0.0001; n harems = 6). However, when the harems were analysed separately, the relationship was present and significant for only some of the harems (RUB96: *rho* = 0.69, P_{10K} = 0.014 – SI196: *rho* = 0.69, $P_{10K} < 0.0001$ – SI296: *rho* = 0.59, $P_{10K} < 0.001$ – SI297: *rho* = 0.58, $P_{10K} < 0.0001$). When the daily number of oestrous females in each harem was considered, the correlation between this value and the daily number of associated males was weak but significant (*rho* = 0.34, P_{10K} = 0.0001, n harems = 6). Yet, the relationship was significant only for SI297 (*rho* = 0.79, P_{10K} = 0.0001) and not for the other harems ($P_{10K} > 0.2$ for each test). When the mean number of males associated with each harem and the seasonal size of the harem (maximum size reached during the season) were considered, the correlation was not significant (*rho* = 0.7, P_{10K} = 0.14, n harems = 6).

III.2.3.2 Mating performance in the harems

The differences in the distributions of the breeding indices among the harems were not significant ($P > 0.7$ in the three cases). In each harem, the holder always made the majority of matings, with a mean of 81% (± 16.5) of the copulations, (med = 80%, $MAD = 4$, $CV = 0.2$, range 50%-100%), and there was no significant correlation between the size of the harem and the proportion of copulations achieved by its holder ($rho = -0.7$, $P_{10K} = 0.102$).

A negative correlation was present between the success of the holder (percentage of total copulations in the harem) and the number of males associated with his harem. The correlation was strong and significant when either the mean number of daily associated males or the seasonal maximum number of males was considered ($rho = -0.94$, $P_{10K} = 0.015$; $rho = -0.86$, $P_{10K} = 0.0196$). The correlation was also present and significant when the total number of different males associated with each harem during the breeding season was taken into account ($rho = -0.84$, $P_{10K} = 0.043$).

The distributions of the indices in each of the seven harems were asymmetric and the variation among individuals large ($CV_{ENFI} = 1.67$ to 2.78, $CV_{FFD} = 1.73$ to 3.72, $CV_{MS100} = 1.41$ to 2.92). The mean of $ENFI$ ranged from 1.75 ± 4.9 to 22.5 ± 31.8, the mean of $MS100$ from 1.108 ± 3.18 to 15 ± 25.1 and the female control index (FFD) had a mean ranging from 33.67 ± 116.63 to 535 ± 753.8 (Table III.4).

	ENFI		FFD		MS100		
Harem	m (– sd)	range	m (– sd)	range	m (– sd)	range	% CO holder (n CO)
RUB96 (12)	1.75 (– 4.86)	17	33.67 (– 116.63)	404	1.11 (– 3.18)	11.1	84 (25)
SF96 (3)	13.0 (– 21.66)	38	250 (– 433.01)	750	14.97 (– 25.1)	43.9	97.7 (44)
SI196 (16)	4.42 (– 12.91)	49	109.57 (– 407.39)	1525	2.76 (– 8.06)	30.6	79 (100)
SI296 (14)	6 (– 14.24)	42	127.43 (– 444.4)	1668	4.10 (– 9.69)	28.7	50 (78)
SM96 (6)	3.83 (– 7.22)	18	77.67 (– 190.24)	466	5.43 (– 10.45)	26.10	80 (15)
SF97 (1)	45	-	1068	-	28.5	-	100 (41)
SI297 (13)	8 (– 22.2)	80	176 (– 625.61)	2258	2.49 (– 6.86)	24.7	76.5 (78)
Overall	5.82 (– 14.92)	80	127.6 (– 419.19)	2258	3.81 (– 9.43)	43.9	81.01 (– 16.51)

Table III.4 Distribution details of the behavioural indices in each harem. Harem name and male sample sizeanalysed in brackets. For each index, mean (– standard deviation) and range are displayed. The percentage of copulations achieved by each harem holder is shown (%CO holder) with, in brackets, the total number of copulations observed in each harem

III.2.4 Paternity analysis

In 1996, paternity was determined for 109 pups out of 115 (94.8% of pups sampled in 1996) at 80% statistical confidence. More than half of the 80% confidence paternities (85 pups, equal to 74% of all the tested paternities) were secure at 95% confidence. Four pups had a father assigned at 78% statistical level of confidence and two had no father assigned. In 1997, a father was found for 74 out of 77 pups (96%) at 80% confidence level and, of these, 66 (86% of pups sampled in 1997) were secure at 95% confidence. Two paternities were assigned at 79% confidence level and one pup had no father assigned. The analyses were conducted separately for the two years and Table III.5 shows the simulation and paternity inference results.

In total, paternity remained unassigned for six pups in 1996 and three in 1997 (4.7% of all paternities). For two of the nine pups, all males were incompatible at two or more loci, while for one pup, one male was mismatching with the mother-pup pair at two loci (i.e. no mismatch with the pup genotype but with the alleles left after matching the pup with the mother and hence supposedly coming from the father). For the remaining six paternities, there was no mismatching and they were assigned at a lower level of confidence. The presence of more than one male compatible with each one of these six pups and the behavioural data consistent with the 'not assigned' fathers, suggest that the markers were not powerful enough to resolve these paternities at the required criterion. It was decided to use only the 80% and 95% confidence paternities following Marshall *et al.* (1998). The paternities assigned at lower level will be discussed later.

Out of the 183 paternities, only two were assigned with a mismatch at one locus between father and pup. In both cases the mismatch was on the locus Hg8.9: it was interpreted as a mutation by the program that assigned the paternities respectively at 95% and 80% confidence level. The probability of non-exclusion had a mean of 0.015 ± 0.026 (range 2.78×10^{-5} - 0.13) in 1996 and of 0.017 ± 0.030 (range 5.36×10^{-5} - 0.18) in 1997. The probability did not differ between breeding seasons (Mann-Whitney: $U = 4795.5$, $P = 0.173$) and gave an average probability of exclusion of 0.985 ± 0.026 (range 0.864-0.999) for both years. The Δ values had a mean of 4.07 (±2.73) and 4.89 (±2.29) and ranges of 0.016-9.58 and 0.030-9.89 respectively for the two years.

	Year 1996			Year 1997		
	80%	95%	Uns.	80%	95%	Uns.
Critical value of Δ from simulation	0.06	1.30		0.05	1.63	
Paternities expected from simulation	94%	61%	6%	93%	60%	7%
Paternities assigned in inference analysis	94.8% (109)	74% (85)	5.2% (6)	96% (74)	86% (66)	4% (3)

Table III.5 Critical Δ scores, percentages of paternity predicted to be solved by simulation and paternity inference results in the two years. The criteria strict (95%) and relaxed (80%) are shown, with the percentage of tests (out of 10000) in which paternity was assigned at the required criterion, and in which no father was identified (Uns.). The proportions of paternities (corresponding number in brackests) assigned at the two confidence levels and for which a father was not identified at the relaxed level are listed in the row below

III.2.4.1 Paternity distribution in the population

Considering only males with at least one paternity assigned (n = 25) the paternity distribution did not differ between years (Mann-Whitney: $U = 84.50$, $P = 0.583$; $n_{1996} = 15$, $n_{1997} = 10$). The same result was also obtained taking into account males with no paternities assigned but which were associated with the harems analysed (Mann-Whitney: $U = 273.0$, $P = 0.525$; $n_{1996} = 29$, $n_{1997} = 17$). Considering both years together, the paternity distribution was very skewed ($g1 = 2.3$) and the inter-individual variation high ($CV = 2.01$), with the majority of males not having any paternity assigned (46%) and a large percentage (35%) achieving only one or two paternities (Fig III.7).

Only a few males had been assigned many more than 10 paternities (6 males out of 25) for a maximum of 32 paternities in the first year and 25 in the second. Considering all males (with and without paternities assigned, n = 46), the mean number of paternities per male was 3.9 ± 8 ($med = 1.0$, $MAD = 1.0$) with an opportunity of selection (I) of 4.04. When only males with paternities were considered, the mean was 7.3 ± 9.7 and I was 1.81.

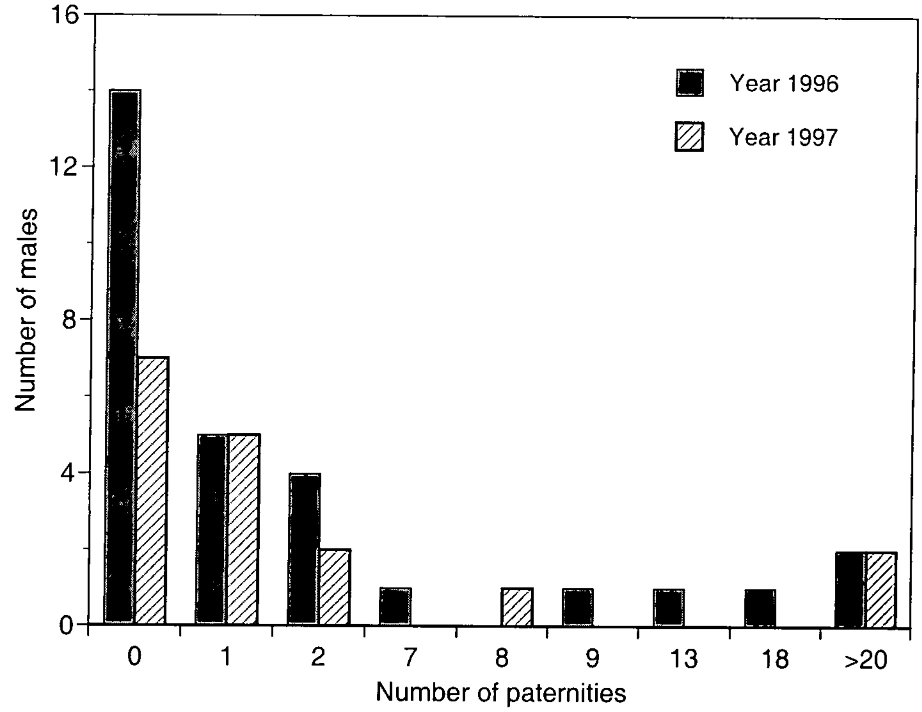

Fig III.7 Distribution of paternities each year in the population. The total number of males was 29 for 1996 and 17 for 1997

Among males that achieved at least one paternity (n = 25), 11 (44%) were harem holders, 11 were secondary and three were tertiary males. All tertiary males had only one paternity assigned. Restricting the data to holders and secondary males, the former had significantly more paternities assigned (Mann-Whitney: $U = 110.50$, $P = 0.0007$; n holders = 11, n secondary = 11). Considering only males with at least one paternity, paternity success was positively correlated with tenure length ($rho = 0.54$, $P = 0.008$), but the variation in tenure explained only 20% of the variance in paternity success ($R^2 = 0.197$, $P = 0.026$).

III.2.4.2 Paternity success in the harems

The distribution of the paternities was asymmetric in each harem ($g1$ from 1.63 to 3.31). Two to nine males accounted for the paternities realised in each breeding unit (Table III.6), with a mean number of paternities per male ranging from 0.86 (± 1.92) to 8.33 (± 15.58). However, the distribution of the paternities was not significantly different among harems (Kruskal-Wallis: $KW = 7.231$, $P_{10K} = 0.288$, $df = 6$). I largely varied across harems and, considering all males (with and without paternities), it had a mean of 5.6 ± 2.9 and ranged from 2.68 to 11.27 (Table III.7)

	Number of males in each harem with/without paternities assigned						
Number paternities	RUB96	SF96	SI196	SI296	SM96	SF97	SI297
0	10	2	11	7	4	1	7
1	1	2	2	6	-	1	5
2	2	-	-	1	1	-	2
7	1	-	-	-	-	-	-
8	-	-	-	1	-	-	1
12	-	-	-	-	1	-	-
18	-	1	-	-	-	-	-
21	-	-	-	1	-	-	-
24	-	-	1	-	-	1	-
32	-	-	-	-	-	-	1
Tot number of males	14	5	14	16	6	3	16

Table III.6 Distribution of paternities in each harem. Number of paternities (left) and the corresponding number of males for each unit are indicated. The total number of males analysed for each harem are shown

In each harem, the holder was assigned the largest proportion of paternities, with a median value of 85.7% (*MAD* = 10.3, range from 56.8% to 96.0). There was no correlation between the number of paternities assigned and either the size of the harem or the total number of males associated with it (*rho* = -0.071, P_{10K} = 0.90; *rho* = -0.52, P_{10K} = 0.242). During the breeding season males other than the harem holder sometimes gained the control of the harem. The period of the turnovers (i.e. the length of time that the new male was able to keep the control of the harem) varied from few hours to few days. To see how these turnovers affected the success of the seasonal harem holders, for each harem the paternities achieved by the 'temporary' holders were summed with the paternities achieved by the seasonal holder. The sum of paternities was larger than those of the seasonal holder in only two harems (SI296: from 56.76% of all paternities in case of one holder to 83.78% for three holders; SI297: from 65.26% of all paternities in case of one holder to 67.34% for two holders). For the other five harems, temporary holders did not achieve any paternity. The success of the harem holder was not significantly correlated with the number of different holders the harem had (*rho* = -0.53, $P = 0.237$), but with the total number of holder transitions (i.e. the number of time the harem control situation changed to another one) (*rho* = -0.82, $P = 0.03$). The proportion of days the principal holder was in control of the females (over the total number of days the harem was held by a male) was not significantly correlated with his paternity success (*rho* = 0.64, $P = 0.133$).

Harem	% pat holder (n pat)	Harem size	Males pat > 0	Mean pat per male (± sd)	*I*
RUB96	58.3 (12)	18	4	0.86 (± 1.92)	4.98
SF96	90.0 (20)	35	3	4.0 (± 7.84)	3.84
SI196	92.3 (26)	55	3	1.86 (± 6.38)	11.27
SI296	56.8 (37)	75	9	2.31 (± 5.35)	5.51
SM96	85.7 (14)	20	2	2.33 (± 4.80)	4.36
SF97	96.0 (25)	40	2	8.33 (± 13.58)	2.68
SI297	65.3 (49)	91	9	3.06 (± 7.96)	6.59

Table III.7 Paternity distribution statistics for each harem: percentage of paternities assigned to the harem holderwith, in brackets, the total number of paternities analysed in the harem; number of males with at least one paternity assigned in the harem; mean number of paternities assigned per male (± standard deviation) and opportunity of selection (*I*)

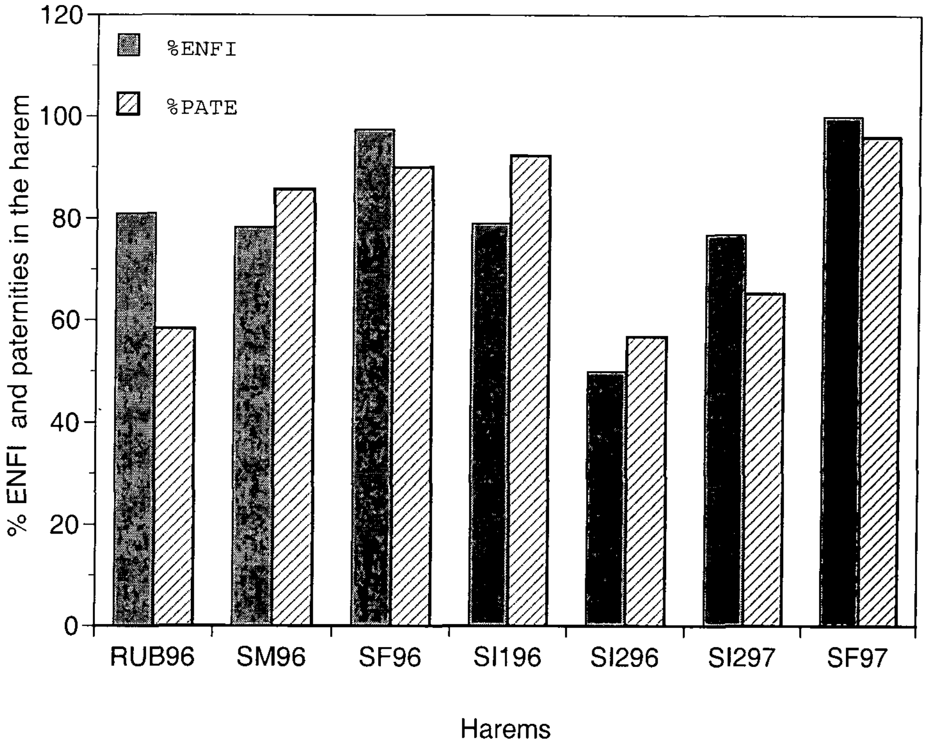

Fig III.8 Percentage of ENFI and paternities achieved by each holder in each harem. Percentages are calculated from 12 paternities and 25 observed copulations for RUB96, 14 and 15 for SM96, 20 and 44 for SF96, 26 and 100 for SI196, 37 and 78 for SI296, 49 and 51 for SI297 and 25 and 41 for SF97

III.2.4.3 Mating performance and paternity

Genetic results confirmed the holder's success in each harem (Fig III.8). The proportion of the paternity's variability explained by the mating success estimates was always very high both among (from 87% to 99%) and within harems (from 79% to 99%). For the harem with the smallest sample size (harem SF97) the relationship was strong for all indices (99%) but it was not possible to test for significance (see Table III.8).

III.2.4.4 Behavioural/demographic indices and genetic data

To each mother/pup pair for which a paternity was found, a father was assigned from the behavioural and demographic data, using different criteria. Seven criteria were considered:

- *HOLDER*: the father was the *harem holder* (seasonal *harem holder*)

- *ASS*: the father was the male associated with the female for the longest period of her presence on land (data from daily records on the female);

	Harems							Years	
	RUB96 (14)	SF96 (5)	SI196 (14)	SI296 (16)	SM96 (6)	SI297 (16)	SF97 (3)	1996 (55)	1997 (19)
ENFI	0.87 *	0.99 §	0.98 *	0.83 *	0.98 §	0.99 *	0.99	0.91 *	0.97 *
FFD	0.79 *	0.95 §§	0.99 *	0.89 *	0.96 §	0.99 *	0.99	0.89 *	0.91 *
MS100	0.87 *	0.99 §	0.98 *	0.84 *	0.99 *	0.99 *	0.99	0.88 *	0.94 *

Table III.8 Coefficients of determination (R^2) between the three behavioural indices and paternities in each harem. Sample size (number of males) for each harem is in brackets. Probabilities were tested using the program R2 (Steiger and Fouladi 1992): * $P \leq 0.00004$, § $0.0006 \leq P \leq 0.00015$, §§ $P = 0.004$. For SF97, the sample size was too small to calculate the significance of the relationships

- *ASS*_E: the father was the male associated with the female for the longest period during her oestrus (data from daily records on the female combined with the estimate of oestrus from date of birth as in Galimberti and Boitani 1999);

- *INT*: the father was the male with the highest number of interactions with the female (data from standard observation periods);

- *CO*: the father was the male with the highest number of copulations recorded with the female (data from standard observation periods);

- *FIR_CO*: the father was the first male seen mating with the female (data from standard observation periods);

- *H_ENFI* the father was the male with the highest *ENFI* in the harem (data from standard observation periods).

The data from these indices were then compared with the genetic results. To find out which index was a better predictor of paternity, each behavioural/demographic father was compared with the genetic assigned father. To have a general idea of congruence between behavioural and genetic results, a single father (*BE_FATH* hereafter) for each mother/pup pair was chosen from the behavioural/demographic data. If the behavioural/demographic fathers were not congruent (i.e. different fathers assigned with different criteria), the male assigned under most criteria was chosen as the behavioural father.

Out of 183 mother/pup pairs for which a genetic father was found, 56% had a father assigned with all seven behavioural criteria. The completeness of the behavioural data was similar in the two seasons (Mann-Whitney: $U = 4254$, $P = 0.484$; $n_{1996} = 109$, $n_{1997} = 74$). There was a mean of 5.5 ± 1.4 criteria filled with a father (minimum number of criteria = 2) and in 70.5% of the cases the criteria were all congruent (i.e. same father for each criteria). In total, the *BE_FATH* for each pup was assigned with a minimum of 50% and a mean of 90.2% (± 15.8%) of all criteria. The general congruence between behavioural and genetic data was very high, and a very large proportion of the paternities assigned to a male with behavioural data was assigned to the same male with genetic markers. The *BE_FATH* was the same as the genetic father 83% of the time in 1996 and 81% in 1997. Considering the behavioural criteria separately, the male assigned from behavioural data and the genetic father were the same individual a mean of 69% to 85% of the time in each harem. The data for each year and harem are shown in Table III.9.

To evaluate how well each behavioural/demographic index was approximating paternity, a regression analysis was run for each index. The genetic paternities that each male had assigned were used as dependent variable and the paternities assigned with each behavioural index were used as independent. The proportion of paternity's variability expressed by the behavioural/demographic indices was always very high, ranging from a minimum of 88.2% for *H_ENFI* and *HOLDER* to a maximum of 96.7% for *ASS*_E ($n = 34$, $P < 0.000001$ in all cases) (Table III.10)

Index	Harems							Years	
	RUB96	SF96	SI196	SI296	SM96	SF97	SI297	1996	1997
BE_FATH	75% (12)	90% (20)	92.3% (26)	73% (37)	85.7% (14)	96% (25)	73.5% (49)	82.57% (109)	81.08% (74)
HOLDER	58.33% (12)	90% (20)	92.31% (26)	56.8% (37)	85.7% (14)	96% (25)	65.3% (49)	75.23% (109)	75.68% (74)
ASS	58.33% (12)	90% (20)	92.31% (26)	62.2% (37)	85.7% (14)	96% (25)	65.3% (49)	77.06 (109)	75.68% (74)
ASS_E	75% (12)	92.9% (14)	92% (25)	79.3% (29)	81.8% (11)	95.2% (21)	76.3% (38)	84.62% (91)	83.05% (59)
H_ENFI	58.33% (12)	90% (20)	92.31% (26)	56.8% (37)	85.7% (14)	96% (25)	65.3% (49)	75.23% (109)	75.68% (74)
INT	50% (12)	78.9% (19)	88.5% (26)	58.3% (36)	66.7% (12)	95.6% (23)	68.2% (44)	69.52 (105)	77.61% (64)
CO	77.78% (9)	100% (11)	93.8% (16)	73.1% (26)	100% (6)	88.2% (17)	76.5% (34)	85.29% (68)	80.39% (51)
FIR_CO	77.78% (9)	100% (11)	93.8% (16)	73.1% (26)	100% (6)	88.2% (17)	76.5% (34)	85.29% (68)	80.39% (51)

Table III.9 Level of congruence between each behavioural index and genetic paternity in each harem and year. The congruence is expressed as percentage of times that the male assigned as a father with one index (indices are on the left) was the same as the genetic father. For each index, the percentage is shown with the sample size in brackets. Index abbreviations are explained in the text above.

	Years		
Index	1996	1997	Overall
HOLDER	0.835	0.917	0.882
ASS	0.893	0.919	0.910
ASS_E	0.952	0.987	0.967
H_ENFI	0.835	0.917	0.882
INT	0.938	0.980	0.954
CO	0.925	0.953	0.941
FIR_CO	0.925	0.953	0.941

Table III.10 Coefficients of determination (R^2)between paternity (as dependent variable) and each index (independent variable) for each year and overall. Sample size was 19 males for 1996 and 15 males for 1997; always $P < 0.000001$

III.4 DISCUSSION

III.4.1 Behavioural estimates of mating distribution in *Mirounga leonina*

Behavioural data from the two years indicated a very high degree of polygyny in the population. The level of mating monopolisation was high, with a large percentage of breeding males that were not able to copulate or to control any female. Out of more than a hundred breeding males, only three had more than 60 copulations assigned and, of these, only one had more than 100 assigned. The mating asymmetry was striking and it was also present among harem holders, supporting the hypothesis of a situation with the majority of males achieving either none or low reproductive success and only a very few showing extraordinary mating performance.

Holding a harem was the most successful mating tactic, in accordance with previous studies on both species of *Mirounga* (Le Boeuf 1974; McCann 1981). Not being able to control a female group strongly reduced the possibility for a male to mate, and among non-harem holders the most rewarding tactic was to be associated with a breeding unit. Only one tertiary male was recorded mating, while 20% of the secondary males achieved at least one copulation.

III.4.1.1 Breeding performance in the harems

A detailed analysis showed no significant difference in the distribution of the mating indices among the harems. The distribution asymmetry and the individual variation in each harem were high, and the holder always achieved the majority of copulations. For the success of the holder, the competition pressure (due to the presence of other males around the harem) seemed to be more important than the 'resource value' (i.e. number of females in the harem). In fact, his success was not correlated with harem size but with the number of associated males. The correlation between harem size and number of associated males was very weak. This suggests that capable holders (in control of larger harems) were able to keep other males distant from their females regardless of harem size. On the other hand, less efficient holders, not able to deter other males, were pressured by their presence and achieved lower success than might be expected based on harem size.

III.4.2 Paternity assignment

The success of paternity inference was very high. A father was found for 95.3% of the pups at 80% confidence level and, out of these, 82% were assigned with 95% confidence (median probability of exclusion 0.997). The results suggest that the level of polymorphism and the parameters used in the analysis were powerful enough to discriminate almost the totality of paternities.

The inference success was similar to that reported for red deer with 84 loci (Slate *et al.* 2000) and much higher than in other paternity analyses of mammal mating systems (Table III.11).

III.4.2.1 Paternities not assigned

Three pups did not have any compatible candidate in the pool sampled and for another six pups it was not possible to assign a father at the chosen 80% confidence level (see Appendix B). There are several possible explanations for the missing paternities. First, it is possible that the actual fathers had not been sampled. This is most likely the case for the three pups with no matching males. For both years, more than 90% of males were sampled each season, but it is possible that some males were missed because they never came to land and that aquatic mating took place. Aquatic mating seems to play an important role in other species of pinnipeds with terrestrial mating, for example in the harbour seal (*Phoca vitulina*: Coltman *et al.* 1998), in the grey seal (*Halichoerus grypus*: Worthington Wilmer *et al.* 1999) and in the Antarctic fur seal (*Arctocephalus gazella*: Gemmell *et al.* 2001). In these analyses, only about 30% of the paternities were assigned, and a very large discrepancy between expected and assigned paternity was reported. Those results supported evidence that aquatic mating may have a much larger importance than it had been previously thought. For the three mother-pup pairs in this study, one female (R409I – season 1996) was seen mating while she was still nursing her pup and before her oestrus period had started. She left the harem on the day her oestrus was estimated to start. As she was still receptive after leaving the island, it is possible that a male intercepted her in the water and fertilised her. The second female (V0404D – year 1996) was seen copulating with the holder of her harem during her oestrus period, but two mismatches were present between that male and the mother/pup

Species	% Pat assigned (n)	Markers used	Inference method/program	Reference
Southern elephant seals (*Mirounga leonina*)	95.3% (192)	9 microsatellite loci	CERVUS (80%confidence)	This study
	74% (192)		CERVUS (95%confidence)	
Red deer (*Cervus elaphus*)	98.2% (172)	84 microsatellite loci	CERVUS (80%confidence)	Slate *et al.* 2000
Red deer (*Cervus elaphus*)	68 (44.1%)	DNA fingerpr. 2-3 proteins	Band sharing	Pemberton *et al.* 1993
Soay sheep (*Ovis aries*)	61.9% (365)	10 microsatellite loci	CERVUS (80%confidence)	Coltman *et al.* 1999a
Soay sheep (*Ovis aries*)	58% (1617)	Combinations of 10 microsatellite loci and 5 allozymes	CERVUS (80%confidence)	Pemberton *et al.* 1999
Harbour seal (*Phoca vitulina*)	8% (275)	6 microsatellite loci	CERVUS (80%confidence)	Coltman *et al.* 1998
	31% (275)		CERVUS (50%confidence)	
Antarctic fur seal (*Arctocephalus gazella*)	19% (184)	8 microsatellite loci	NEWPAT	Gemmell *et al.* 2001
Grey seal (*Halicoerus grypus*)	35.7% (233)	9 microsatellite loci	NEWPAT	Worthington Wilmer *et al.* 1999

Table III.11 Success of paternity inference in mammal mating systems. Species, percentage of paternities assigned out of the total paternities tested, markers and methods used are indicated genotypes, so the paternity was not assigned. For the third female (V2462D – year 1997), no data on mating behaviour were available, since she was a very difficult female to observe. She was always in the very centre of the harem (likely trying to avoid any contact with the holder) and no interactions between her and either the holder or any other male were recorded throughout the breeding season. Also the year before conceiving the pup she behaved atypically. In fact, in 1995 she was in the harem for only four days and, most important, she left the beaches without giving birth. This would support the hypothesis that aquatic mating can sporadically take place in this species.

A second possible explanation for the missing paternities could be a lower efficiency in fertilisation by particular males. Of the three females above, two were seen copulating with the same male (SILVIO). From his appearance and behaviour, this male was estimated to be extremely old, and in some species sperm efficiency declines with age. Unfortunately, data on elephant seal (or other pinniped species) sperm are not available, because of their mating behaviour and the difficulties in manipulating the animals make them very difficult to obtain.

A third possibility is that more loci are needed to better discriminate some of the paternities. Mutation and null alleles could be present and should be considered in paternity inference (Marshall *et al.* 1988). From the total number of loci screened in this analysis, it was chosen to allow only one mismatch. Screening more loci and allowing the presence of mutations or null alleles at more than one locus would likely improve the results of the analysis (Slate *et al.* 2000). This would also possibly solve the six cases where a father was assigned only at lower confidence (78-79%). It was decided not to accept these paternities as true in order to have consistent genetic results to analyse and compare with behavioural data. However, for all six paternities, the behavioural father was assigned without mismatches and was either the most or the second most likely genetic father (Δ between the first and the second father within 1% of the mean critical Δ value). It is very likely that for these paternities the analysis was not powerful enough to discriminate a father among candidates with similar genotypes.

III.4.3 Paternity and polygyny

The genetic data confirmed the behavioural results that the distribution of paternities was very asymmetric in the population, with the majority of males having no or one paternity assigned. The inter-individual variation was high, and there were only very few males with more than 20 of paternities assigned. The variance in paternity success in the population was very similar to the variance estimated with behavioural indices, and gave an opportunity of selection of 4.04, which denotes an elevated level of polygny (Boness *et al.* 1993). A significant correlation was present between the reproductive success and the period a male stayed on land, but the tenure length explained only a small proportion of it.

III.4.3.1 Paternity success in the harems

The paternities were very unevenly distributed within harems. There was variation in the paternity distributions among the breeding units, but it was not significantly different. In each harem the holder was assigned the highest proportion of paternities (median 86%, maximum 96%). As for the behavioural estimates, his paternity success was not related to the harem size and efficient holders were able to control large numbers of females. In contrast, the holder success was related to the number of holder transitions, which can be considered as an index of harem stability. Some harems go through splitting, joining and taking-overs, and these factors can strongly influence the holder's success. The opportunity of selection differed among harems (from 2.68 to 11.27), showing that the level of polygyny can vary locally, and in some harems reached a value three or four-fold greater than the smallest value.

Comparison with other Mirounga populations

The proportions of paternities achieved by the holders at SLI were higher than those reported for the population of Punta Delgada on Peninsula Valdés, Argentina (Hoelzel *et al.* 1999). In five harems at DEL (harem size: 30-119; total paternities per harem: 5-19; males tested per harem: 2-8) the holders achieved 50% (n = 6) to 70% (n = 10) of the paternities in each harem (overall mean = 58%) (Hoelzel *et al.* 1999). The range in harem size was similar to the range analysed on SLI (18-91 females; total paternities per harem: 12-49) and, from the comparison of these two studies, the strategies adopted by non-holder males seem to be more rewarding in the population of DEL than in the population of SLI. Different factors can influence the paternity patterns within harems. The holder's capacity for controlling a harem and keeping the other males distant, and the social and topographic environments are all fundamental aspects that can affect the likelihood of mating monopolisation among males.

The physical and behavioural resource holding potential (*RHP*) of a holder (i.e. body size, dominance rank and fighting experience) strongly influences his capacity for controlling females (Haley *et al.* 1994; Modig 1996). Moreover, the variation in *RHP* between the holder and other males defines the likelihood that a holder can dominate over other males and therefore monopolise the mating context in the harem. Differences in size between holder and non-holder males were greater in the SLI population during the seasons from 1995 to 1998 than in the population of DEL during the 1993 and 1994 seasons (Fabiani 1996; Galimberti and Fabiani unpublished data). As a likely consequence, on SLI harems had a significantly lower number of associated males than on Punta Delgada (median = 0.212 vs 0.692 – Galimberti *et al.* 2000a), and non-holder males achieved 22% vs 41% of the paternities (n paternities = 183 for SLI and n = 50 for Delgada). Hence, holders at Sea Lion Island more efficiently manage the reproductive context, effectively keep the other males far away from the harems and achieve a significant higher reproductive success (Fisher exact test: $Phi = 0.205$, $df = 1$, $P = 0.0059$).

The topographic environment was also different between the two analysed colonies of southern elephant seals. Although the density of females is higher on SLI than on DEL (111 vs 62 female/km^2 – see Galimberti and Boitani 1999; Baldi *et al.* 1996), the tidal range differs between the two colonies. At DEL the tidal range is very large, during low tides the beaches become much deeper and departing females have to cover much longer distances to reach the sea. This is not the case at SLI, where the tidal range is small and the distance between a harem and the sea is, on the whole, short. As a consequence, a smaller percentage of female departures are intercepted on SLI, departure events are shorter and less frequently involve interaction with secondary males (Galimberti *et al.* 2000a). Hence, it is possible that this is one of the environmental factors that influence the potential for polygyny in the population, and that females intercepted during departure are effectively fertilised by secondary males. However, no genetic data are so far available to support and quantify the hypothesis that males copulating with departing females successfully inseminate the females.

In northern elephant seals (NES) harem holders report a mean low success despite dominating mating. In seven harems of 32-224 females (total paternities per harem: 7-18; number of males per harem: 3-15), the proportion of paternities achieved by each holder ranged from 26.7% (n = 15) to 80% (n = 10) (Hoelzel *et al.* 1999). In the study of Hoelzel *et al.* (1999) SES holders were in general more successful than NES holders. The authors suggested that success lower than expected could be in part due to the recent bottleneck that the NES have recently gone through. However they also suggested that different behaviours adopted by either males or females at the two locations and topographical features could account for the higher success in the southern species. Females at NES harems mate more frequently and with more males than females in SES harems. Similarly, the number of secondary males encountered by departing females may be greater for the northern species.

Northern and southern elephant seals have a very similar mating system; nevertheless differences are present both between the two species and between the two SES populations. In both species, only a few males monopolise seasonal matings; however, the range of monopolisation varies among populations, and the pattern of male success are influenced by phenotypic, behavioural and environmental factors. In this context, the northern species seems to represent the lower end of the variation in polygyny level recorded in the genus *Mirounga*. On the contrary, populations of southern elephant seals show higher levels of polygyny, with the seals of SLI exhibiting a level of polygyny that can be considered as the most extreme in the range analysed.

III.4.4 Estimating paternity from behavioural data

III.4.4.1 Breeding performance

The results showed that the behavioural indices of individual breeding performance used were all good predictors of relative male reproductive success in the harems, with coefficient of determination (R^2) in the range 0.79-0.99. Both the mating (*MS100* and *ENFI*) and the female control (*FFD*) estimates approximated well to the paternity distribution in each harem, but sometimes the fertilisation and mating indices worked better than the female control estimate.

III.4.4.2 Behavioural/demographic data

Fathers assigned to each mother/pup pair with behavioural data accurately identified the true father in each harem. The level of congruence was always high, the two fathers being the same a minimum 58.3% of the time (as *HOLDER*) and a maximum of 100% (*FIR_CO*). The effectiveness of the behavioural indices measured with coefficient of determinations was high for all the indices (from 0.88 to 0.99). The most effective were those that were less easy to collect, because related to the history of each female and not to the history of each breeding unit. Therefore, the indices *FIR_CO* or *ASS_E* gave better predictions than the index *HOLDER*, and for the first two it is necessary to follow each female during the breeding season (i.e. the data are from observation periods), while for the third one it is enough to record the harem holder during population censuses. Nevertheless, recording the holder of each harem very well approximated the paternity success variance in the population ($R^2 = 0.88$). Further more, it was as effective as identifying the male with the highest *ENFI* in the harem, which requires much more laborious data collection.

III.4.4.3 Discrepancy between behaviour and genetics

The behavioural/demographic indices did not completely describe the paternity distribution in the population, as each one failed to explain a small part of the assigned paternities. This could indicate that different strategies were adopted by either the males or the females in the population, or that genetic inference should have been more powerful. Often, non-holder males are very close to the harems, always trying to get matings with the females. Sometimes, a harem holder during season, the correspondence *HOLDER* vs genetics would increase from 75 to 80% and *FIR_CO* vs genetics from 85 to 93%. If the second fathers were chosen for those paternities, the holder success would also increase and the paternity distribution in the harems would be more congruent with each harem's history. For RUB96, the success of the holder would change from 58% to 78%. Two of the paternities not assigned to the seasonal holder would be to the holder of the neighbour harem. This male was dominant to the RUB96's holder, the two harems were very close and he was seen moving to RUB96 and mating with females in the group. For SI296, the percentage of paternities assigned to the seasonal holder would increase from 57% to 65%. This harem was less stable than the others, and during the breeding season it experienced 10 holder transitions. The seasonal holder held the harem for only 55% of the 57 days that the harem lasted. Two other males held the harem for 22 days in total and achieved copulations with the females in the group. If the 24 paternities assigned to the seasonal holder were added to the 10 assigned to the other two holders, they would represent 92% of the total paternities in the harem. For SI297, the holder success would increase from 65% to 69%. Also in this case, the small increase would be congruent with the history of this harem. It was a large group and it split into two smaller harems with two different holders (one being the holder of the initial big harem). Sixty percent of the paternities not assigned to the seasonal holder of SI297 were assigned to the holder of the newly-formed harem. Hence, taking into account the paternities of both males, they would represent 87% of the total paternities in the harem. Finally, for the harem SF96, if the second most likely father was chosen for the paternity assigned by CERVUS with one mismatch (pup V3602I – see Appendix B), the holder would be assigned all but one paternity in his harem and his success would increase from 90% to 95%. This male was very successful in keeping other males distant (max daily number of associated males = 2, see Table III.3) and controlling the females of his harem. Hence, his elevated relative reproductive success would reflect his performance during the breeding season.

These results strongly support the hypothesis that each harem represents an almost independent breeding unit, defined by its social-demographic context. This is characterised by the number of females, the number and personality of peripheral and holder males and it strongly influences the distribution of paternities within the breeding group.

IV. Patterns of relatedness within the colony

IV.1 INTRODUCTION

In classical models of population genetic structure, individuals mate randomly within and between sub-populations. However, in some species, individuals associate in groups within which mating and social behaviours may produce non-random mating patterns within and between groups. In this context, social structuring may provide an additional hierarchical level at which genetic variation may be maintained and within which behaviours may minimise inbreeding (Sugg *et al.* 1996).

In many species, the identity, social status and relatedness of individuals can play an important role in determining the outcome of behavioural interactions, which often have reproductive consequences. The interaction of social, ecological and demographic factors determines the reproductive opportunity available to an animal and hence the strategies that an animal can pursue and their relative efficiencies (Dunbar 1984). The importance of this socio-ecological context is most evident and widely studied in species that form permanent or semi-permanent groups and long-term relationships (e.g. in *Lycaon pictus*: Girman *et al.* 1997; *Macaca fascicularis*: De Ruiter and Geffen 1998; *Microcebus murinus*: Radespiel *et al.* 2001). Nevertheless, social context is also likely to be important for species that form short-term aggregations (Pomeroy *et al.* 2001; Burland *et al.* 2001). For example, breeding success can be influenced by the time an individual arrives at the breeding site and the time it leaves it. Breeding success can also be affected by the individual's position within a breeding group and, further, by the other individuals forming the aggregation.

Female harassment by males in pinnipeds is common (McCann 1981; Campagna *et al.* 1998; Le Boeuf and Mesnick 1990a,b; Boness *et al.* 1995). Intense harassment can reduce maternal performance and female reproductive success (Le Boeuf and Mesnick 1990a; Boness *et al.* 1995). One female strategy of harassment defence is the synchronisation of breeding (i.e. clustering of females during reproduction), so that females that breed at the same time and in the same place can benefit from the dilution effect of the presence of other females. Land breeding polygynous pinnipeds have concentrated breeding seasons (Riedman 1990, Boyd 1991) and females that breed out of the breeding peak (the central phase of the breeding season) are exposed to higher levels of harassment (Boness *et al.* 1995, Galimberti *et al.* 2000a) and can, as a consequence, show lower breeding success. In these circumstances, the breeding timing for a female is likely to influence her breeding performance and hence her reproductive success.

IV.1.2 Philopatry and site fidelity

Social organization of a species can result in non-random matings when one, or both, sex exhibits strong philopatry (i.e. returns to the natal colony to breed) (Chesser 1991a). In many mammals, females are the philopatric sex and males tend to disperse for breeding (Greenwood 1980). Female philopatry and site fidelity (i.e. return to the same breeding colony in consecutive seasons) can result in high relatedness between female members of a social group, and high relatedness may in turn lead to a greater co-operation between group members. At the same time, female philopatry coupled with male dispersal can result in differing degrees of genetic relatedness within and between sexes (Surridge *et al.* 1999).

Southern elephant seal females become sexually mature at 3-4 years of age and may have a reproductive lifespan of 21years (Hindell and Little 1998). Males reach social reproductive maturity not before 9-10 years old and live on average up to 16 years (McCann 1985), with a documented maximum of 21 years (Arnbom *et al.* 1992). If a high level of philopatry is present in elephant seals, an overlap of generations would be likely to happen. In this case, depending on the degree of spatial and temporal synchronisation, female philopatry and site fidelity would result in the presence of related mothers at the breeding site or within the breeding groups (harems). At the same time, site fidelity of males, together with their longevity and potential breeding life span, would lead to the possibility of repeated matings with either the same or related partners.

Mark-recapture studies have reported site fidelity in both female and male elephant seals, with males tending to disperse more than females (see Discussion in Chapter V). On Sea Lion Island, females exhibit a high level of site fidelity (Fabiani *et al.* in prep.). Furthermore, they display within-colony site fidelity, returning to the same part of the colony during consecutive seasons. From 1996 to 1999, out of 646 females that returned to the island for two to four breeding seasons, 72% (464) returned to the same breeding zone of the colony each year, and a mean of 37% (236) to the same breeding area (see Chapter II for zone and area definitions). In contrast, males showed a more flexible strategy in choosing the breeding site, coming back to the same colony but less frequently to the same zone or area within it. In this case, of 84 males, 62% came back each season to the same zone of the island, and only 29% to the same area.

IV.1.2 Female and male movements within the colony of SLI

Females on SLI may change harem between arrival and parturition, but no females have ever been recorded moving between harems after giving birth. The only exception can be isolated females that, after giving birth in unsuitable places, normally move to reach the closest harem and remain there for the rest of their breeding season (Galimberti *et al.* 2000a). On the other hand, males may either frequent a particular area of the colony or move among different harems and zones during the breeding season (see map of the breeding colony in Fig II.2). Individual movements within the colony could to a certain degree be affected by the relatedness patterns among seals on the island. If this was the case, the level of relatedness could influence the way in which the colony develop and therefore could also have effect on the individual reproductive success (i.e. through seal distribution and mating opportunities).

IV.1.3 Aims

In this chapter the level of genetic relatedness among the seals in the colony was assessed:

a) the accuracy of the relatedness estimate was determined by analysing pairs whose relationship was known. For this analysis, both seals from Sea Lion Island and Elephant Island were considered;

b) kinship was assessed among seals of SLI to investigate whether levels of genetic relatedness within-colony reflected site fidelity and philopatry previously identified for this species;

c) relatedness was considered among different status-categories of males (holders and secondary males) to see if any pattern of relatedness was present among males of different status.

IV.2 RESULTS

IV.2.1 Relatedness estimates from known relationships

To assess the accuracy of Queller-Goodnight R values in estimating relatedness between individuals of unknown relationship, average R values for pairs of known relatedness were calculated. As a sample of unrelated individuals ($R = 0$), adult seals from SLI ($n = 263$) were compared with seals belonging to the EI colony ($n = 46$). Mother-offspring pairs ($n = 192$) were identified from behavioural data (and confirmed by parentage analysis), while father-offspring pairs ($n = 186$) were identified based on the parentage analyses (see Chapter III). Both sets of pairs were predicted to show relatedness values of 0.5. Maternal half-siblings (R expected value $= 0.25$) were available from 30 females that bred both years in the study harems. Paternal half-sibling pairs were identified for each harem in both years (from 8 to 35 half-sibs in each harem), for a total of 1647 pairwise comparisons. Values of relatedness for mother-offspring dyads did not differ from those from father-offspring so they were pooled together. The same procedure was followed for the half-sibling dyads (Mann-Whitney: $U > 20000$, $P > 0.9$ both cases). Parental-offspring pairs with a predicted relatedness of 0.5 (n dyads = 378) had a mean R value of 0.52 ± 0.14 (SE = 0.007; 95% CI = 0.51, 0.54), while the mean R value for half-sibling pairs (expected value: 0.25) was 0.34 ± 0.2 (SE = 0.005, 95% CI = 0.33, 0.35, n dyads = 1677). The mean relatedness for known unrelated individuals (expected value: 0.0) was 0.004 ± 0.22 (SE = 0.002, 95% CI = 0.0002, 0.007, n dyads = 13088). The three distributions strongly differentiated among each other (for each pairwise comparison: Mann-Whitney: $U > 48500$, $P < 0.0001$).

Although a Kolmogorov-Smirnov test revealed significant deviation from the expected normality for two distributions (unrelated: *KS* = 0.013, *df* = 13088, *P* = 0.000 – half-sibs: *KS* = 0.036, *df* = 1677, *P* = 0.000 – parent-offspring: *KS* = 0.030, *df* = 378, *P* = 0.2), the analysis of skewness and kurtosis indices (unrelated: *kurtosis* = -0.2, *skewness* = 0.1 – half-sibs: *kurtosis* = -0.2, *skewness* = -0.3) and of normality plots showed that deviation was slight. In both distributions, the deviation was mostly due to a set of outliers at the upper end of the range of *R* values (Fig IV.1). For the distribution of unrelated individuals, 68.5% of the values were within one standard deviation of the mean; for the half-sibling distribution, 50.7% of the values were included within one standard deviation and 69% were within one standard deviation for the parent-pup distribution.

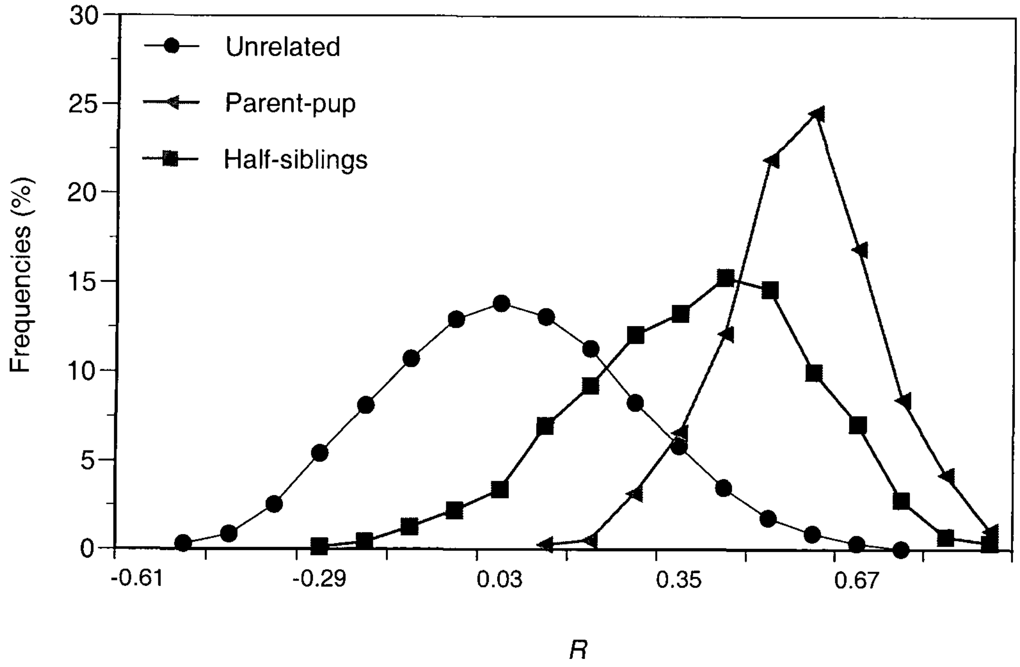

Fig IV.1 Distributions of relatedness values (*R*) of known unrelated, parent-offspring and half-sibling pairs (see text for distribution details)

The three distributions were partially overlapping. However, comparing the distribution of parent-offspring and that of half-siblings, it was possible to determine the probability of misclassifying a dyad to a particular relationship. According to Blouin *et al.* (1996), the midpoint between the means of the two distributions (which in this case is 0.43) can be used as a cut-off value for the classification of the dyads.

A dyad with $R \leq 0.43$ would be included in the distribution of half-siblings, whereas a dyad with $R > 0.43$ would belong to the full-sibling distribution. The percentage of the randomly generated half-sibs that fall to the right of the cut-off value would represent the type I error rate, while the percentage of parent-offspring that fall to the left of the cut-off value would be the type II error rate. In this case, an expected percentage of 34% parent-offspring or full sib pairs would be misclassified as half-sibs, while 22% of half-sib dyads would be classified as full sibs or parent-offspring. The overlapping region between these two distributions and that of unrelated individuals was smaller. The cut-off point between unrelated and half-sib relatedness distributions was 0.17, with an expected 18% of half-sibs classified as unrelated and 22% of unrelated seals classified as half-sibs. For the unrelated and parent-offspring distributions, 3.7% of parent-offspring dyads would be misclassified as unrelated and 11% of unrelated individuals would be classified as having a level of relatedness of 0.5 (cut-off point between the two distribution 0.26). Although the distributions were not completely separated, the data should not negate their use to deduce related dyads from not-related dyads in the population with an acceptable level of confidence (see De Ruiter and Geffen 1998; Kays *et al.* 2000; Lucchini *et al.* 2002).

IV.2.2 Kinship assessment in the population

The mean R value for all sampled seals of SLI was 0.077 (± 0.232, n dyads = 18528) in 1996 and 0.074 (± 0.233, n dyads = 9453) in 1997. There was no difference between the two years (Mann-Whitney: $U = 88204516$, $P > 0.3$). However, when males and females were considered separately, relatedness was slightly different between the two years both for males (Mann-Whitney: $U = 2878272$, $P = 0.005$) and females (Mann-Whitney: $U = 9871980$, $P = 0.022$).

Each season, the mean relatedness level among females was higher than mean relatedness level among males. However, the difference was significant in 1996 (Mann-Whitney: $U = 10867933$, $P < 0.0001$) but not in 1997 (Mann-Whitney: $U = 2751021$, $P > 0.1$). Males were more related to females than among each other, but, as above, the difference was only significant in 1996 (1996: Mann-Whitney: $U = 14228197$, $P < 0.0001$ – 1997: Mann-Whitney: $U = 4311455$, $P = 0.841$). Details of relatedness values calculated among females, males and between males and females are given in TableVI.1

	1996			1997		
	mean (± sd)	n	range	mean (± sd)	n	range
FF vs FF	0.094 (± 0.231)	115	-0.59 – 0.90	0.080 (± 0.238)	77	-0.72 – 0.82
MM vs MM	0.051 (± 0.228)	78	-0.65 – 0.77	0.069 (± 0.228)	62	-0.52 – 0.78
MM vs FF	0.074 (± 0.234)	193	-0.64 – 0.95	0.072 (± 0.231)	139	-0.71 – 0.83
All	0.077 (± 0.232)	193	-0.65 – 0.95	0.074 (± 0.233)	139	-0.72 – 0.83

Table IV.1 Estimates of relatedness in the population of SLI. Mean *R* values (± standard deviation), number of individuals (n) and range are given among females, among males, between females and males, among all seals each year

IV.2.3 Genetic structure among harems and areas

To investigate if the distribution of the harems on the island had some influence on the genetic pattern of the population, analysis at zone and area levels were carried out on both females and males. A zone was a stretch of beach separated from other zones by stretches of habitat (rocks or cliffs) not suitable for elephant seals and not used for breeding, while an area was a part of the beach clearly delimited by topographical features but not separated from adjacent areas by unsuitable habitat. Each area may include one or more harems, and each zone included two or more areas.

Females sampled for this genetic study belonged to the same zone. Females belonging to four areas were studied in 1996, and females belonging to two areas in 1997. In only one area there were two well distinct harems, while all the others comprised one harem (in one case a single harem split in two at the end of the season). Considering the females breeding in the two harems (SI196 and SI296) of the same area (SI96), the level of relatedness within a harem was no higher than the level of relatedness within the area (Mann-Whitney: U = 1252922, P = 0.92; n dyads of same harem = 1131; n dyads of same area = 2211).

Males sampled in this study comprised individuals frequenting the three zones and all the areas and harems on the breeding colony. Each year there were 11 areas and 11-13 harems on the island. Relatedness among males frequenting the same zone was no higher than among males frequenting different zones (Mann-Whitney: U = 2924167, $P = 0.9$ n dyads of same zone = 2145; n dyads of different zone = 2628). As for the females, relatedness among males within harems was no more elevated than among males within areas (Mann-Whitney: $P > 0.1$ each year: 1996: n dyads of same harem = 164; n dyads of different harem = 1012 – 1997 1996: n dyads of same area = 187; n dyads of different area = 716).

R estimates of male-female dyads were analysed to investigate the relatedness between females breeding in a particular area and males that frequented that area. Mean genetic relatedness between females and males within the same area was not significantly different from the mean relatedness between females and males coming from different areas. In 1996, estimated relatedness within areas was lower than relatedness between areas (0.06 ± 0.24 vs 0.08 ± 0.23; Mann-Whitney: $U = 6043567$, $P = 0.0009$) while in 1997 the opposite tended to be true (0.08 ± 0.23 vs 0.07 ± 0.23) but the difference was not significant ($P = 0.643$). Investigating relatedness between males and females at harem level, females were not significantly more related to males associated to the same harem than to males frequenting different breeding units ($P > 0.1$ each year).

IV.2.4 Relatedness within and between harems

IV.2.4.1 Females

The seven harems were analysed in details to test the hypothesis that females belonging to the same harem were more closely related than females belonging to a different harem. For each harem, the distribution of the *R* estimates between females within the harem was compared with the distribution of *R* values between females belonging to that harem and females belonging to other breeding groups. When the seven harems were considered together, the average values of relatedness within harem of 0.095 (± 0.23) was not significantly greater than the value between harems of 0.087 (±0.23) (Mann-Whitney: $U = 20174059$, $P > 0.1$). When the two years were considered separately, the relationship was significant only for 1997 (1996: Mann-

Whitney: $U = 7606236$, $P > 0.9$ – 1997: Mann-Whitney: $U = 2287866$, $P < 0.0001$). The distributions are illustrated in Fig. IV.2.

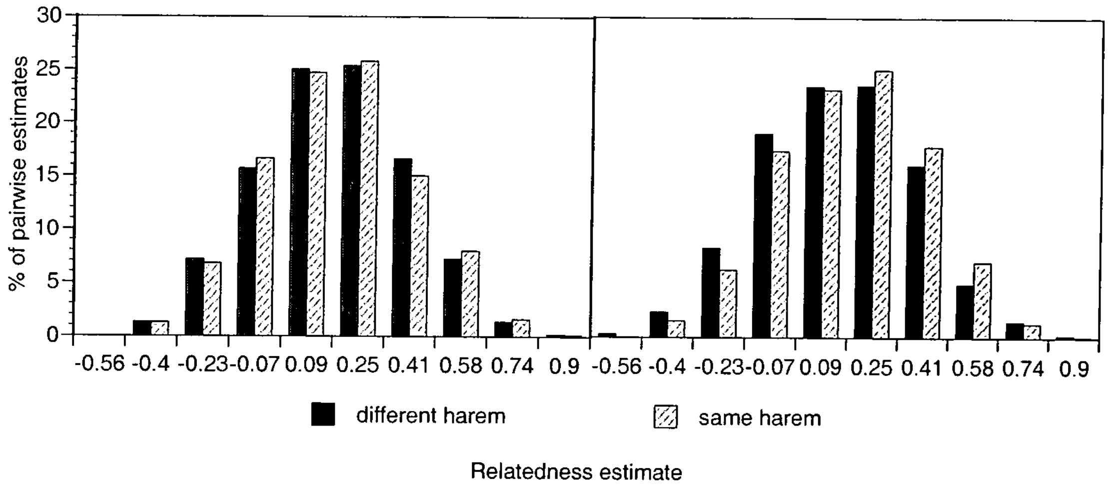

Fig IV.2 Relatedness distribution among females within and between harems, each year. Mean of 0.095 ± 0.23 and 0.093 ± 0.23, respectively for 1996 (n dyads same *harem* = 1504, n dyads different *harem* = 10102); mean of 0.095 ± 0.23 and 0.062 ± 0.23, respectively for 1997 (n dyads same *harem* = 1600, n dyads different *harem* = 2652)

When the single harems were analysed, the distribution of within-harem relatedness largely varied among them (Kruskall-Wallis: $H = 36.896$, $df = 6$, $P < 0.0001$) (Fig IV.3). The mean level of relatedness among females within a harem was not related to the size of the harem ($rho = 0.5$, $P = 0.56$). For six out of seven harems, relatedness within a harem was not higher than relatedness between harems. Only for the harem SI297 females breeding in the group were more closely related (Mann-Whitney: $U = 940752$, $P < 0.0001$). Details are in Table IV.2.

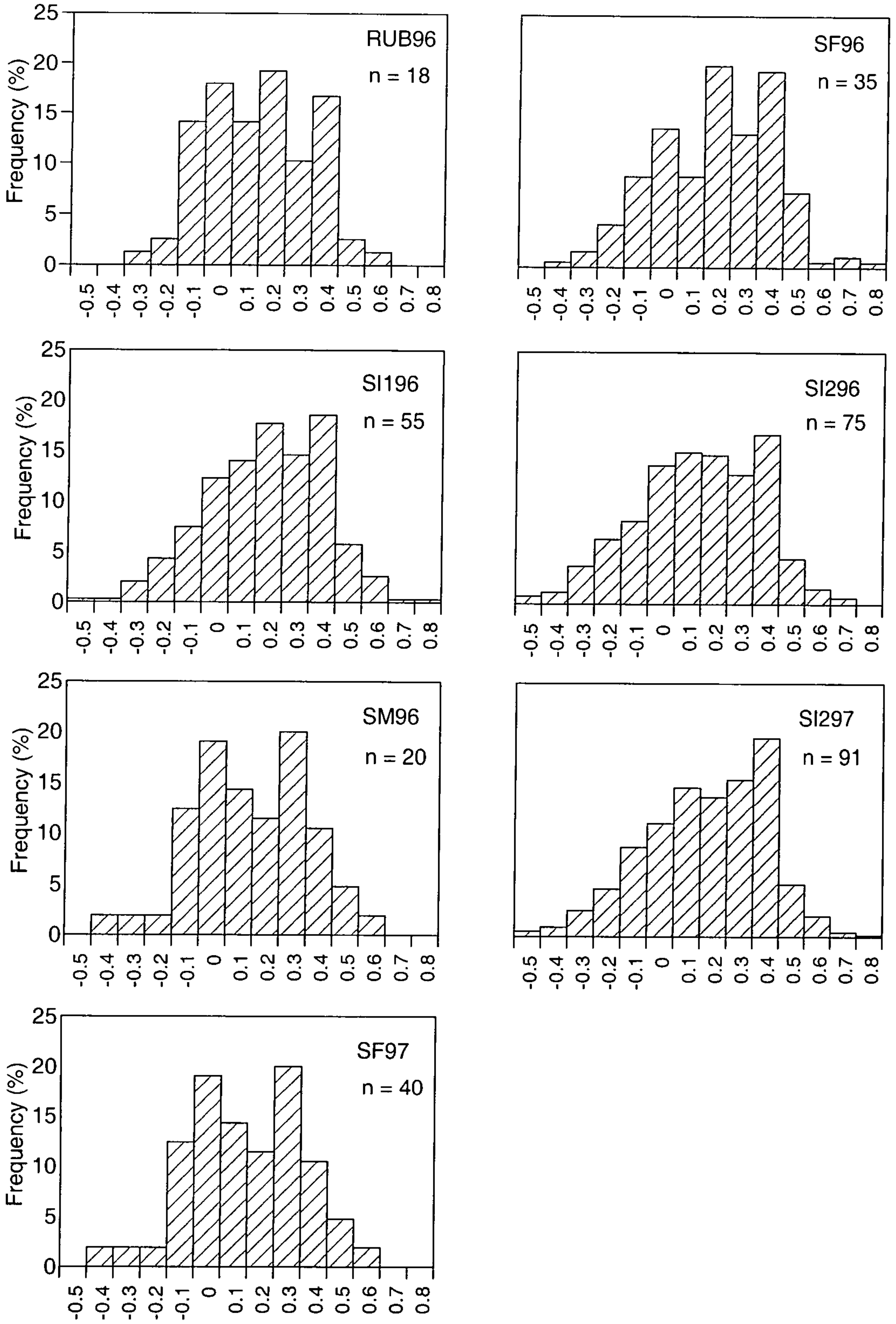

Fig IV.3 Distribution of relatedness values (*R*) for female pairs in each harem. The number of females breeding in each harem is shown (n)

	mean R (± sd)	n dyads	min R	max R
RUB96	0.08 (± 0.20)	78	-0.37	0.61
	0.09 (± 0.23)	1326	-0.59	0.83
SF96	0.13 (± 0.23)	190	-0.42	0.75
	0.10 (± 0.23)	1900	-0.57	0.90
SF97	0.04 (± 0.23)	325	-0.48	0.71
	0.06 (± 0.24)	1326	-0.72	0.79
SI196	0.12 (± 0.22)	351	-0.53	0.76
	0.09 (± 0.24)	2376	-0.57	0.83
SI296	0.08 (± 0.24)	780	-0.59	0.71
	0.09 (± 0.23)	3000	-0.59	0.90
SI297 *	0.11 (± 0.23)	1275	-0.55	0.82
	0.06 (± 0.24)	1326	-0.72	0.79
SM96	0.07 (± 0.21)	105	-0.45	0.60
	0.09 (0.23)	1500	-0.57	0.76
All	0.10 (± 0.23)	23104	-0.59	0.82
	0.09 (± 0.23)	12754	-0.72	0.90

Table IV.2 *R* values of females within and between harems. For each harem, the first row shows details for *R* calculated between the females within the harem; the second row shows details for *R* calculated between the females of the harem and all other females belonging to different harems. Mean (± standard deviation), number of dyads analysed, minimum and maximum *R* values are given. Asterisk indicates the harem for which the two distributions of *R* values were significantly different

IV.2.4.2 Females, harem holder and secondary males

To test the hypothesis that females select genetically distant mates, the level of kinship between the harem holder and the females breeding in his harem was investigated. Relatedness between each female of a harem and its holder was compared with relatedness values between those females and the males associated with the harem. Mean *R* values between females and the holder varied among harems (from -0.046 ± 0.17 for SI196 to 0.2 ± 0.19 for RUB96 – Details in Table IV.3). The difference between female-holder relatedness and females-peripheral male relatedness was not consistent among the breeding groups. For three harems (RUB96, SI296, SI297) females were significantly more related to their harem holder than to peripheral males ($P < 0.009$ for each harem). For the other two harems (SI196, SM96) the opposite was true and females were more related to secondary males than to the holder. However, for SM96 the difference was not significant ($P >$ 0.07).

	mean *R* (± sd)	n dyads	min *R*	max *R*
RUB96	0.20 (± 0.19)	14	-0.25	0.44
SF96	-0.008 (± 0.15)	20	-0.30	0.24
SF97	-0.03 (± 0.18)	26	-0.39	0.40
SI196	-0.04 (± 0.17)	27	-0.35	0.32
SI296	0.18 (± 0.29)	40	-0.51	0.95
SI297	0.19 (± 0.22)	51	-0.29	0.55
SM96	-0.03 (± 0.18)	15	-0.41	0.32

Table IV.3 *R* values of female-harem holder pairs are given for each harem. Mean (standard deviation), number of dyads analysed, minimum an maximum *R* values are given

When relatedness of female-holder pairs (*fh* pairs) was compared with relatedness of female and holders of the other harems (*foh* pairs), the results were not consistent among harems and differences were not always significant. For three harems (RUB96, SI296, SI297) *fh* relatedness was higher *foh*, but the difference was significant only for SI296 (Mann-Whitney: $U = 9042$, $P = 0.0079$; n *fh* pairs = 40, n *foh* pairs = 360) and for SI297 (Mann-Whitney: $U = 17622$, $P = 0.0061$; n *fh* pairs = 51, n *foh* pairs = 561). For the other four harems, females in each group were more related to males controlling other harems than to their holder. However, the difference was significant for SI196 (Mann-Whitney: $U = 4406$, $P = 0.0035$; n *fh* pairs = 27, n *foh* pairs = 243) but not for SM96, SF96 and SF97 ($P > 0.05$).

IV.2.4.3 Holder and peripheral males

Each harem had a principal holder and 1-14 associated males (see Chapter II for definitions and behavioural observations). The hypothesis that males tend to associate with a harem controlled by genetically related holders (and consequently that a harem holder allows related males to mate in his harem) was tested. The level of relatedness between the principal holder of each harem and the males associated with that harem was investigated, comparing the *R* values between holder and associated males with those between holder and males not associated to his harem. No significant differences were found for any of the breeding units (Mann-Whitney: $U > 170$, $P > 0.1$ for each harem). The relatedness distributions were very different among harems, as mean *R* values ranged from 0.037 (±0.25) to 0.19 (± 0.25). Results did not exhibit consistency among either harems or years; holders were more related to peripheral males in some harems and less related in others. Details are shown in Table IV.4.

Relatedness was also assessed between each holder and his peripheral males that had at least one paternity assigned (see Chapter III for paternity assignment). The *R* values between holders and secondary males of their harem that achieved at least one paternity were compared with *R* values between holders and secondary males of their harems that did not report any paternity. For each harem, two to four secondary males were identified as genetic fathers of pups born in the harem. The level of relatedness between those males (n = 21) and their respective harem holder was not significantly different from the level of relatedness between the holders and the other peripheral males of the harem (Mann-Whitney: $U = 461.5$, $P = 0.112$, n = 35).

Harem holders were not significantly more related among each other than other males in the colony. The degree of relatedness among harem holders showed a mean of 0.059 ± 0.247 (n dyads = 18) in 1996 and of 0.16 ± 0.24 (n dyads = 3) in 1997. In both years, the values were not significantly different from the level of relatedness among non-holder males (Mann-Whitney: $U > 3200$, $P > 0.4$ each year; 1996: mean = 0.049 ± 0.23, n dyads of non-holders = 2556 - 1997: mean = 0.074, n dyads of non-holders = 1711). However, the results should be taken with caution due the difference in the size of the holder and non-holder samples.

	mean *R* (± sd)	n dyads	min *R*	max *R*
RUB96	0.08 (± 0.29)	11	-0.28	0.63
	0.11 (± 0.18)	66	-0.31	0.58
SF96	0.19 (± 0.25)	3	-0.97	0.35
	-0.4 (± 0.22)	74	-0.45	0.52
SI196	-0.22 (± 0.25)	13	-0.39	0.49
	-0.31 (± 0.19)	64	-36	0.58
SI296	0.13 (± 0.21)	13	-0.20	0.51
	0.19 (± 0.26)	64	-0.52	0.73
SI297	0.08 (± 0.29)	12	-0.17	0.37
	0.11 (± 0.18)	48	-0.40	0.43
SM96	0.04 (± 0.25)	5	-0.35	0.34
	0.01 (± 0.21)	72	-0.47	0.49

Table IV.4 For each harem, *R* values between holder and peripheral males (first row) and between holder and males not associated to his harem (second row) are shown. Mean (± standard deviation), number of dyads analysed, minimum an maximum *R* values are given. The two distributions were not significantly different for any of the harems

IV.2.5 Relatedness among seals showing site fidelity

Each year, it was possible to identify seals observed breeding on SLI for the first time and seals that had been on the island during previous breeding seasons. The level of relatedness for seals showing at least two years of site fidelity was investigated and compared with relatedness for seal at their first breeding attempt on the island. Both in 1996 and in 1997, the level of relatedness between females never seen breeding on SLI (*first year females*) was significantly higher than between females showing breeding fidelity to the island (*returning females* - Mann-Whitney:

$U > 206219$, $P < 0.002$ each year; 1996: *first year females*: mean = 0.16 ± 0.23, n = 34; *returning females*: 0.067 ± 0.23, n = 81 – 1997: *first year females*: mean = 0.10 ± 0.24, n = 24; *returning females*: 0.073 ± 0.29, n = 53). Among males, the results were not consistent between seasons. In 1996, males present in the previous year were significantly more related than those recorded as breeding on the island for the first time (Mann-Whitney: $U = 255165$, $P = 0.0027$; *returning males*: mean = 0.06 ± 0.22, n = 50; *first year males*: mean = 0.03 ± 0.24, n = 28), while there was no difference between the R values in 1997 ((Mann-Whitney: $U = 65407$, $P > 0.75$).

IV.2.5.1 Within-colony site fidelity and relatedness

Among those females that returned from the previous year (81 in 1996 and 47 in 1997), 30 came back to breed in the same harems. In 1996, two and 10 females returned to SI296 and SF96, respectively and, in 1997, 14 females returned to SI297 and four to SF97. Out of the 30 females, three came back to the same harems for three consecutive seasons (from 1995 to 1997). Considering the two years together, 22 females came back to harem SI2 and five to SF (females returning from 1995 to 1997 were considerate only once). The mean level of relatedness among returning females within the same harem was 0.135 ± 2.56 (n dyads = 10) for SF and 0.098 ± 0.224 (n dyads = 210) for SI2, and there was no significant difference in the relatedness of returning individuals between the two harems (Mann-Whitney: $U = 1155$, $P = 0.593$). For SI2, among the total number of dyads, 41 (19.5%) exhibited an R value larger than 0.3, and eight (3.8% of the total) an R larger than 0.5. Based on the relatedness distributions in the population (see IV.2.1) an R value larger than 0.3 could indicate a pair of related seals (cut off point between unrelated and half-siblings distributions is 0.17), while an R value larger than 0.5 could indicate a related pair of half-siblings or parent-offspring (since the cut off point for half-siblings and parent-offspring distributions was 0.43). For harem SF, 3 dyads out of 10 (33%) showed relatedness level higher than 0.3 and only one higher than 0.5.

Among returning females that had a level of relatedness equal to or larger than 0.4 (n pairs = 14), five pairs (35.7%) shared an allele at each locus. Nine pairs showed an R value larger than 0.5, and of these only two pairs (22%) shared an allele at each locus. Four pairs (44%) shared an allele at all but one locus, and three pairs did not share any allele at two loci. In total, seven pairs of returning females matched an allele at each locus, and the matching would suggest first-order relationship. However, none of these returning females were described as primiparous females, hence no speculation on the relationship between them was possible based on observational data (i.e. it was not possible to indicate if they were mother-daughter or full-sib relatives). Details on returning female pairs that showed a level of relatedness larger than 0.4 and shared one allele at each locus are in Table IV.5.

ID1	ID2	Years they bred in the same/diff location:	*R* value
A655I (3, 3)	V0472I (4, 2)	2 yrs in the same area 1 yr in different zones	0.64
R551I (5, 2)	V0126I (3, 2)	3 yrs in the same harem	0.52
V0461I (6,3)	V0710I (6, 4)	5 yrs in the same harem 1 year in same area (adjacent harems)	0.40
R470D (4, 3)	V0710I (6, 4)	2 yrs in the same harem 2 yrs in the same area	0.41
R551I (5, 2)	V2139I (5, 3)	4 yrs in the same harem 1 yr in the same area	0.40
V0130I (5, 2)	V0472I (3, 2)	2 yrs in the same harem 1 yr in the same area	0.46
V0209I (6, 3)	V0710D (6, 4)	2 yrs in the same harem 4 yrs in different zones	0.45

Table IV.5 Returning females with high level of relatedness and one shared allele at each locus. The table shows: the identity of each female and, in brackets, the number of seasons that she was breeding on SLI and the number of different harems she belonged to; the number of years that the two females were observed breeding in the same or different location; their *R* value

When only females belonging to the same harem were considered, those that came back from previous seasons were significantly less related among each other than females never seen breeding on SLI (Mann-Whitney: $U = 231725$, $P = 0.0004$; *returning females*: mean = 0.08 ± 0.22, n dyads = 1471–*first year females*: mean = 0.13 ± 0.23, n dyads = 278). However, the mean *R* values were significantly different for 1996 (Mann-Whitney: $U = 53058$, $P = 0.0010$) but not for the 1997 season (Mann-Whitney: $U = 62386$, $P > 0.09$). Details are in table IV.6.

	1996		1997	
	mean (± sd)	n dyads	mean (± sd)	n dyads
First year females	0.15 (± 0.23)	121	0.12 (± 0.23)	157
Returning females	0.07 (± 0.23)	739	0.09 (± 0.23)	732

Table IV.6 Relatedness for females belonging to the same harem for each season. Mean *R* values (± standard deviation) are indicated between females recorded breeding for the first year on SLI and for females returning from previous seasons. n = number of dyads analysed for each category

To determine if returning females were more closely related to females breeding in the same harem than to those from other groups in the colony, the genetic relationship between returning females and those breeding in the same harems was assessed. If the two years were considered together, returning females (n = 132) showed higher level of relatedness with females breeding in the same harem than with females breeding in different harems (Mann-Whitney: $U = 2433102$, $P = 0.005$; mean = 0.08 ± 0.22 vs 0.06 ± 0.23). However, the difference was significant in 1997 (Mann-Whitney: $U = 262688$, $P = 0.0003$, n = 51) but not in 1996 (Mann-Whitney: $U = 939135$, $P = 0.49$, n = 81). Details are in Table IV.7.

When the same analysis was carried out among returning males, no significant differences in relatedness were found between returning seals and those breeding in the same harem or area, either in 1996 or in 1997 ($P > 0.3$ each one).

	1996		1997	
	mean (± sd)	n dyads	mean (± sd)	n dyads
Same harem	0.073 (± 0.225)	739	0.093 (± 0.225)	731
Different harem	0.066 (± 0.228)	2501	0.05 (± 0.229)	646
Same area	0.064 (± 0.23)	1261	-	-
Different area	0.069 (± 0.27)	1979	-	-

Table IV.7 Mean *R* values (± standard deviation) between returning females and those breeding in the same or different harem (or area). n = number of dyads analysed for each category. Results from area and harem in 1997 were the same (see text)

IV.3 DISCUSSION

IV.3.1 Estimating relatedness from pairwise comparisons

Data generated by the seven microsatellite loci investigated in this study appeared to give a useful resolution for relatedness assessment. A relatedness estimate can only be obtained if there is a set of unrelated individuals as a reference population. Individuals between the study and the reference populations need to be genetically different because they are unrelated only, and not because they have been sampled from different, isolated populations that have diverged over time (Blouin *et al.* 1996; de Ruiter and Geffen 1998). In this context, the Elephant Island colony was an ideal reference population for measuring relatedness among seals of SLI (i.e. populations were moderately differentiated at both microsatellite and mitochondrial markers - see Chapter V).

The mean *R* values of known relationships well approximated the expected values of unrelated pairs (SLI vs EI seals averaged 0.0) and parent-offspring (0.52), though half-sib pairs averaged 0.34, higher than the expected 0.25. A possible explanation would be that half-sibs are also related through either their mothers or fathers (de Ruiter and Geffen 1998). Females in the colony reported an average relatedness of 0.09 while males of only 0.05. However, males with assigned paternities (see Chapter V) showed a higher level of mean relatedness (0.1). This, together with female relatedness, could account for the over-estimation of the relationship among half-sibs pairs, since 0.25 + (1/2 x 0.1) + (1/2 x 0.09) = 0.345, which is equal to the *R* mean value exhibited by the half-sib pairs (de Ruiter and Geffen 1998).

The higher relatedness among males identified as fathers could be related to the paternity assignment. In this case, either the number of loci or their polymorphism could be not high enough to provide the level of resolution needed in the paternity analysis. In some harems, a few paternities were genetically assigned to males other than the 'behavioural fathers' (for example for the harems RUB96 and SI296 – see Discussion of Chapter III). In these paternities, the 'behavioural father', who was also the harem holder, was assigned by CERVUS as the second most-likely father without mismatches. This means that those males recognised as 'genetic fathers' (and used in the calculation of relatedness of paternal half-sib pairs) showed genotypes very similar to those of the harem holders, which in turn were assigned the majority of the other paternities in the harem. Because of this genotype similarity among some males recognised as 'genetic fathers', the half-sibs would show a higher level of relatedness than the expected value of 0.25. This was also confirmed from the relatedness analysis of fathers and holders. In the first case, males identified as 'genetic fathers' were on average more related than other males in the colony (0.085 ± 0.25) while harem holders were not more closely related among each other than the other males (0.059 ± 0.25).

These results suggest that the number of loci may be large enough to distinguish between half-sib and parent-offspring pairs (Goodnight and Queller 1999) but their level of polymorphism (number of alleles per locus = 4 -9, mean = 6.9) may not be sufficient. A study on the brown-headed cowbird (Alderson *et al.* 1999) overcame the same kind of problem by doubling the number of loci. Therefore, increasing the number of loci would also increase the sensitivity of these relatedness analyses. Nevertheless, after assessing the degree of overlap between the distributions (unrelated, half-sibs and parent-offspring), the microsatellites provided an adequate tool to classify unrelated and related pairs of seals.

IV.3.2 Colony relatedness

The populations of SLI and EI showed a level of relatedness comparable to that expected from unrelated individuals ($R = 0.0$). Moreover, the level of relatedness among seals from SLI was significantly higher than the level of relatedness for SLI-EI dyads. This result indicates a first level of distinction, on a geographic scale, between the two colonies. Site fidelity has been documented for other populations of this species (Lewis *et al.* 1996; Nicholls 1970; Hindell and Little 1988). In this context, the higher level of relatedness within each colony would support the idea of site fidelity at colony level, as indicated by a mark-recapture study on the island (Fabiani *et al.* in prep.).

IV.3.2.1 Within colony relatedness

Results of relatedness within the population were not consistent either among years or among harems. Yet, it was possible to identify some pattern of relatedness among the seals of the island.

In elephant seals, as in other pinnipeds (Twiss *et al.* 1994), both males and females show site fidelity to their colony. Males tend to forage over shorter distances (McConnell and Fedak 1996; Campagna *et al.* 1999) and, at the same time, they may disperse more than females. In this context, seals from the same colony are expected to show some level of relatedness and females should be more related than males. In both years, females from SLI exhibited a higher level of relatedness among each other than males (0.09 vs 0.05 and 0.08 vs 0.07), although the difference was only significant for 1996. This result would be preliminary evidence that males also come back to the same colony to breed, but the difference of relatedness within sex would strengthen the evidence that females disperse less than males to breed. Fig IV.4 shows the mean relatedness values in the population, including comparisons among males and among females.

If seals on the island exhibited a certain degree of relatedness, then relatives were distributed randomly throughout the colony rather than being confined in the same breeding group or area. For both males and females, level of relatedness did not increase from areas to harem. Further, males did not exhibit higher relatedness with males (or females) from the same breeding unit than with males (or females) from a different part of the colony. When each harem was analysed separately, within harem relatedness values were on average higher than between harems for only one out of the seven harems (harem SI297). For the other harems, within relatedness was sometimes but not always larger than between harems, but the difference was never significant. It is important to underline that the patterns of kinship within harems strongly differed among harems (see Fig IV.3). Seven different breeding contexts were analysed, though only in one of them was a pattern in the relatedness distribution recognised. This seems to support the proposition that closely related females are present in the colony (3-4% of the female-female dyads had a values higher than 0.5 each year; n dyads = 11606 and 4252) but they do not tend to cluster together. A different situation was recognised in the grey seal

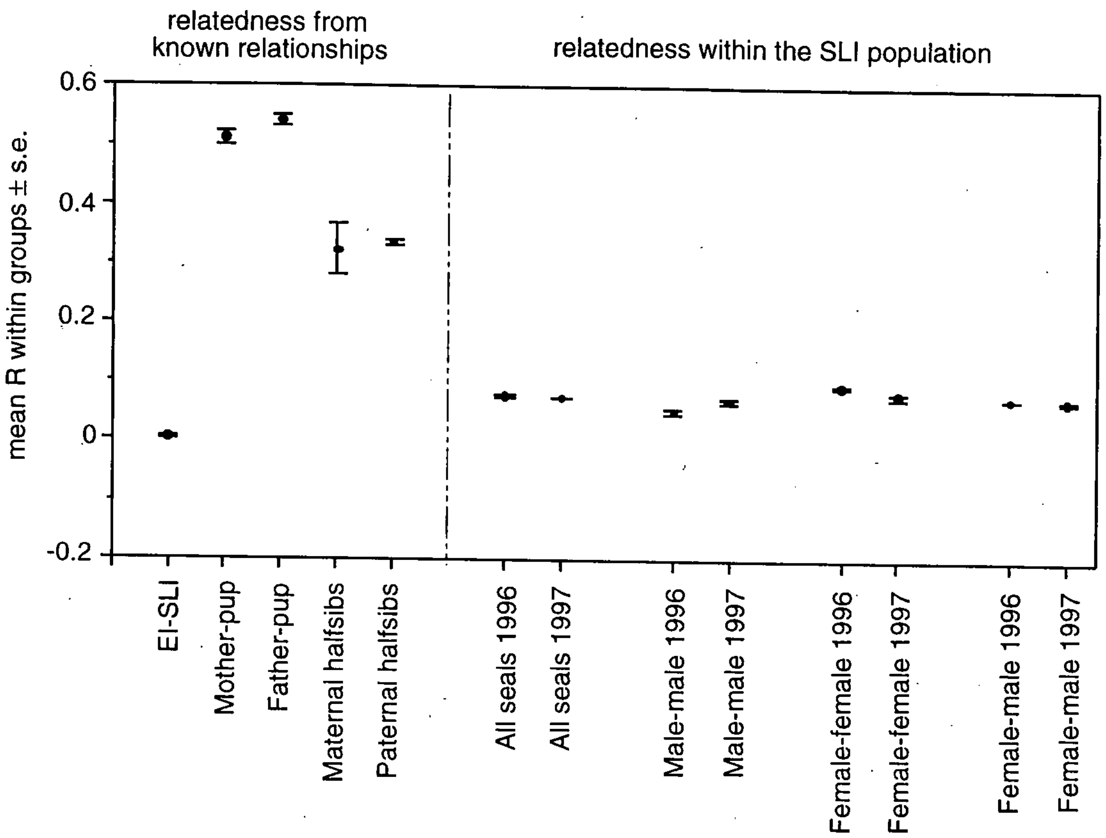

Fig IV.4 Mean relatedness values (– standard error) of known and unknown relationships in the population

(Pomeroy *et al.* 2001). For the colony of North Rona, the analyses of relatedness among females of one breeding season detected a fine-scale genetic structuring within the breeding colony, and locations where on average females had higher than average relatedness to the colony were found. In this case, however, it was also possible to identify proximate benefits associated to those sites of the colony. They occurred close to access points and had particular topography characteristics (access to water, flat terrain), resources that likely influence the female reproductive performance (Twiss *et al.* 2000). On the contrary, elephant seal females on SLI breed on a homogeneous habitat (i.e. uniform sandy beaches with no relevant topographical features) and all harems are very close to the sea.

Previous kinship studies on pinnipeds concerned the assessment of relationships among females (Shaeff *et al.* 1999; Pomeroy *et al.* 2000, 2001) or the identification of pattern of paternal relatedness within a breeding colony (Worthington Wilmer *et al.* 2000). In this study, genetic relationships among seals were assessed to investigate any correspondence between genetic and social structure. When the relationship between the females belonging to a harem and its holder was analysed, no consistent results were found. In some harems, females were more closely related to the holder than to other males frequenting the harem while, in some others, the opposite was true. Similar results were obtained when the analysis was restricted to harem holders only; relatedness between females and the holder of the harem was not consistently different from that with other harem holders. It was not possible to detect any clear pattern of genetic relatedness between females and those males that achieved the highest level of reproductive success in the colony. Neither there was evidence that females tended to mate with genetically diverse males (Amos *et al.* 2001).

In species that are organised in long-term social groups with only one male (the alpha) responsible for most of the paternities in the group (e. g. in *Lycaon pictus*: Girman *et al.* 1997; *Canis lupus*: Lucchini *et al.* 2002), females are usually related to each other (often they also belong to the same matriline) but not to the alpha male. On the other hand, subordinate males appear to be relatives and to have higher level of relatedness with the alpha male. Kinship with a dominant member of the group in case of the wild dog is the prerequisite of pack membership and also provides some reproductive value to subdominant males (Girman *et al.* 1997). In southern elephant seals of SLI, females do not show any pattern of relatedness with the male they are most likely to mate with (the harem holder). Similarly, secondary males that occasionally succeed in mating with females of a harem, and that are also subordinate to the harem holder (Fabiani 1996), are no more related to the holder than to other males. In this species, males do no participate in rearing the offspring and no co-operation has been documented among females of the same group. In a study on reproductive strategies of SLI females carried out during the same time as this study (Galimberti *et al.* 2000b), it was found that females tended to prefer larger harems at their arrival. In a sample of 205 females that settled in one harem at arrival and then moved to another harem for parturition, 70% shifted to larger harems. The grouping of females during the breeding season under the control of a single dominant male reduces the likelihood of encountering secondary males and reduces some of the short- and long-term breeding costs for the female (Le Boeuf 1991). The lack of any relatedness pattern between females and their harem holder in this population seems to suggest that male phenotype (and genetic relatedness) does not affect a female in choosing her breeding group (Galimberti *et al.* 2000b). Females seem to adopt the strategy of clustering in both time and space essentially to avoid high level of harassment and lower breeding success (Boness *et al.* 1995). They do not seem to adjust their harem choice on the phenotype of males that either control or frequent it.

IV.3.3 Female and site fidelity

This study identified general low mean *R* at SLI and found that the majority of individuals within a harem were no more related to each other than to individuals from different harems (Fig IV.5).

The mark-recapture study on this population from 1995 to 1999 reported both site fidelity to the colony and a tendency to return to the same part of the island during consecutive seasons (Fabiani *et al.* in prep.). Out of 646 females that came back to the island to breed in two to four consecutive seasons, a mean of 72% returned to the same zone each year, and 37% returned to the same area. Eight-four per cent of returning females gave birth within an average of 500 m from the

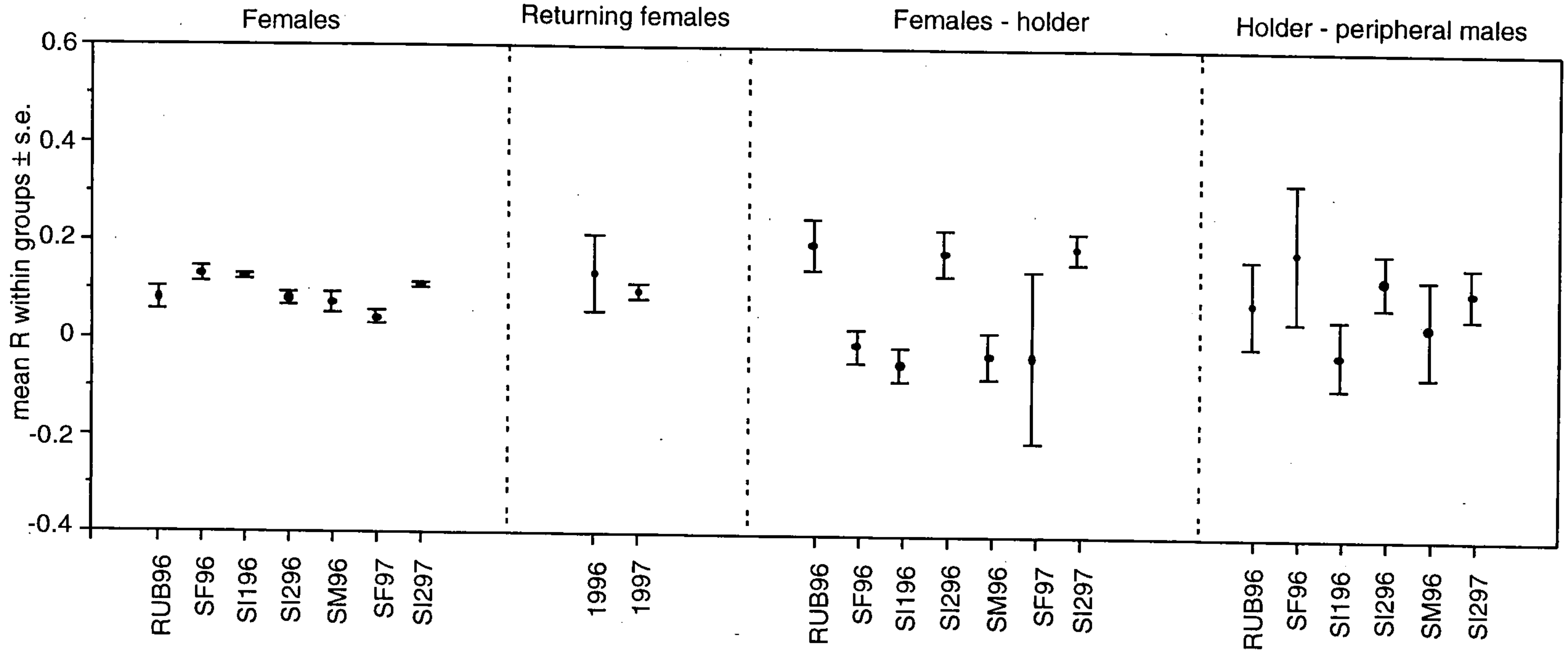

Fig IV.5 Mean relatedness values (– standard error) among different groups of seals within harems previous year location, although only 8% (n = 142) came back to the same area every year for five consecutive seasons. The SLI population displayed a level of site fidelity comparable to levels of site fidelity found in other pinnipeds (Table IV.8), and higher than that recorded for the population of Peninsula Valdés (Lewis *et al.* 1996).

When the level of genetic relatedness between females that came back to the same harem was investigated, *R* averaged 0.1 each year. Twenty percent of the dyads of females within the same harem (n dyads = 220) showed an *R* value larger than 0.3 but only 4% had an *R* value larger than 0.5. Furthermore, returning females show a level of relatedness lower than that showed by females breeding on the island for the first time. In both years, the mean relatedness of females breeding for the first year on SLI was higher than the mean relatedness of returning females. Data on female philopatry of the study population (Fabiani *et al.* in prep.) showed that 63% of the females born in 1995 (n = 38) came back to the same zone to breed in 1999. In this context, a higher level of relatedness among females at their first breeding attempt could be the result of those females being born in the same harem and, therefore, having the same father, i. e. the holder of the harem where their mothers were breeding.

Among harbour seals of Sable Island, females showed high fidelity to the breeding site but only a small percentage (30% out of 36) returned within 500 m of their previous pupping site, and related females seemed distributed randomly along the beaches (Shaeff *et al.* 1999). Results from this study seem to support the evidence that site fidelity and philopatry do not strongly affect the genetic structure of the colony. Even if females tend to come back to the same part of the island to reproduce, the size of the harems are still sufficient to dilute the average relatedness within them. As a consequence, the mating system of this population does not seem to lead to any genetic sub-structure within the colony.

Species	n	Site fidelity	Habitat	Mating system	Reference
Southern elephant seal (*Mirounga leonina*)	398	86% within 3 km of previous breeding site	Land	Harem defence polygyny	Lewis *et al.* 1996
Southern elephant seal (*Mirounga leonina*)	646	84% within 0.5 km of previous breeding site 100% within 3 km of previous breeding site	Land	Harem defence polygyny	Fabiani *et al.* (in prep.)
Northern elephant seal (*Mirounga angostirostris*)	150	70% within 4 km of previous parturition site	Land	Harem defence polygyny	Reiter *et al.* 1981
Grey seal (*Halichoerus grypus*)	59	76% within 80 m of previous pupping site	Land	Scramble competition polygyny	Pomeroy *et al.* 1994
Harbour seal (*Phoca vitulina*)	36	30% within 0.5 km of previous pupping site	Land	Scramble competition polygyny	Schaeff *et al.* 1999
Weddel seal (*Leptonychotes weddelli*)	94	59.5% in the same colony as previous year (km scale)	Ice	Territory defence polygyny	Stirling 1974
Antarctic fur seal (*Arctocephalus gazella*)	115	80.2% return at least once to the same beach (440 m^2)	Land	Territory defence polygyny	Lunn and Boyd 1991
South australian fur seal (*Arctocephalus forsteri*)	15	93.3% in the same colony as previous year (km scale)	Land	Territory defence polygyny	Stirling 1971
Northern fur seal (*Callorhinus ursinus*)	7940	78% in the same central breeding area (up to 1 km long)	Land	Territory defence polygyny	Gentry 1998
Australian sea lion (*Neophoca cinerea*)	29	69% within 5 m of previous pupping site	Land	Territory defence polygyny	Higgins and Gass 1993

Tab IV.8 Female breeding site fidelity in different species of pinnipeds. Sample size (n), type of breeding habitat and mating system are indicated

V. Population genetic structure

V.1 INTRODUCTION

Marine mammals have a high capacity for dispersal and inhabit an environment with few geographic barriers. Pinnipeds, extremely well adapted to marine habitats, are however still tied to land (or ice) for reproduction and moulting, and geographical range, philopatry, dispersal potential and mating system are likely to be very important factors in defining the genetic structure of the populations.

V.1.1 *Mirounga leonina* genetic structure

Mirounga leonina breeding colonies are concentrated on sub-Antarctic islands near the Antarctic convergence. They are divided into three main stocks (i.e. group of close colonies within each ocean), defined as the South Georgia stock (south Atlantic ocean), the Kerguelen stock (south Indian ocean) and the Macquarie Island stock (south Pacific ocean) (Laws 1994). The South Georgia stock is numerically the largest and includes, among other colonies, South Georgia (SG), South Shetland Islands, Falklands and the colony of Peninsula Valdés (PV, Argentina). Heard Island (HD) is part of the Kerguelen stock while Macquarie Island (MQ) is part of the Macquarie stock (Fig V.I)

Inter-population studies on southern elephant seals began with Lydekker (1909) who analysed skull characteristics and proposed three subspecies – *falclandicus*, *crosetensis* and *macquariensis* – corresponding to the types found in the South Atlantic, southern Indian and South Pacific, respectively. However, Bryden (1968) explained some morphometric differences among those populations as environmentally determined (Slade 1997). In a study on allozymes, Gales *et al.* (1989) found significant differences between the Macquarie and Heard Island colonies at four allozyme loci. Slade (1997) and Slade *et al.* (1998) confirmed the differentiation between these two colonies for both mitochondrial and microsatellite markers. They also found significant differentiation at mitochondrial DNA but not at microsatellite markers between HD and SG. When the Peninsula Valdés colony was compared with the other colonies at mitochondrial DNA, the differentiation was

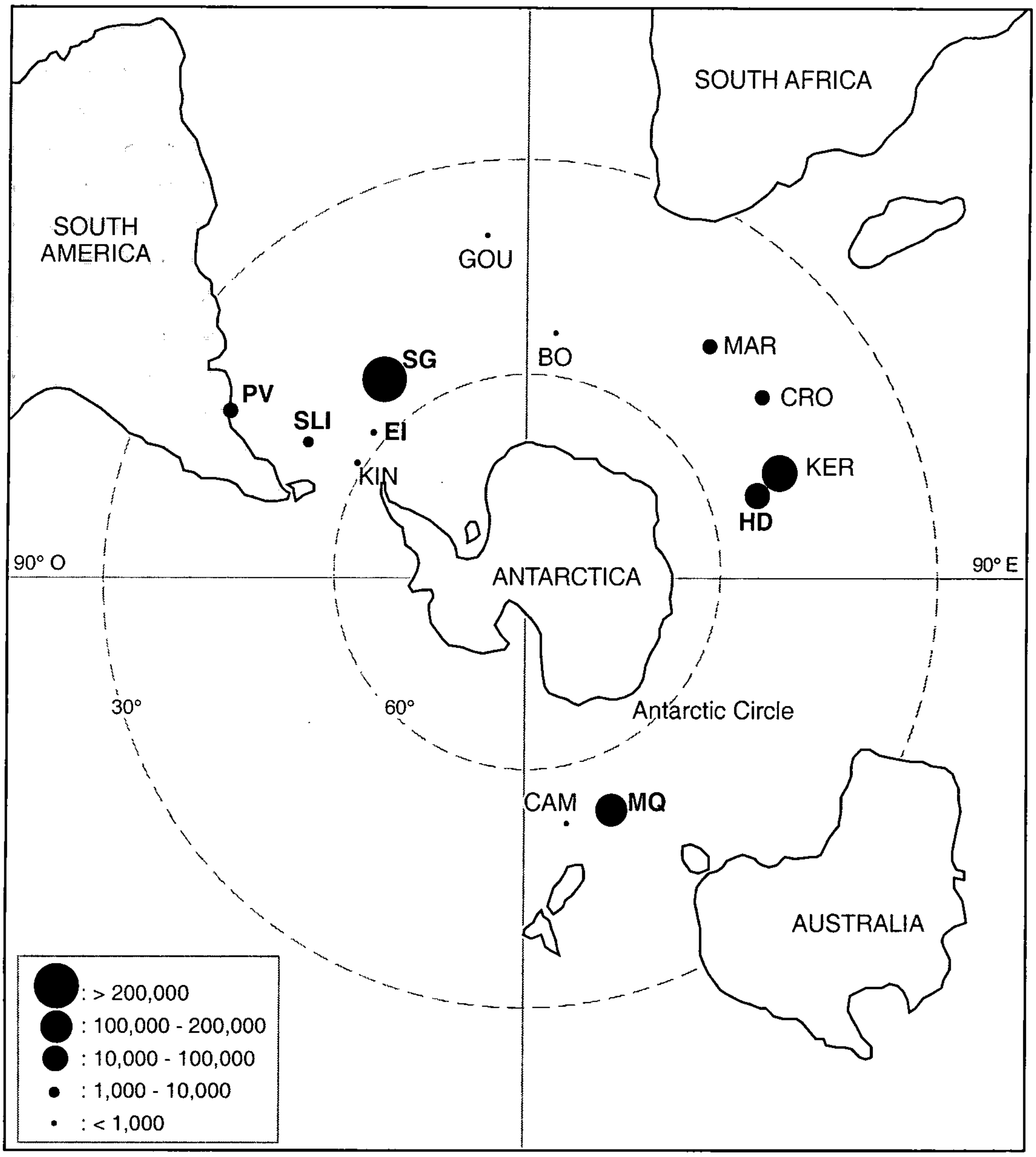

Fig V.I Position and size of *Mirounga leonina* colonies. The studied colonies are indicated in bold and are: PV = Peninsula Valdés, SLI = Sea Lion Island, EI = Elephant Island, SG = South Georgia, HD = Heard Is., MQ = Macquarie Is. Other colonies shown: KIN = King George Is., GOU = Gough Is., BO = Bouvet Is., MAR = Marion Is., CRO = Isles Crozet, KER = Kerguelen, CAM = Campbell Is.

always very high. Therefore they suggested a common origin (separation estimated at the last ice-age, 18 000 years ago) and a divergence time of MQ and PV populations 200 000 – 300 000 years ago. In a more recent study (Hoelzel *et al.* 2001), PV and SG were analysed in more detail at both microsatellite and mtDNA loci, and morphological characters were also compared between the two populations. Differences between SG and PV were supported at both genetic and morphometric measures, and they were genetically confirmed also when compared with data from populations from MQ and HD. Haplotypes from MQ and PV formed separate and very distinct monophyletic lineages, while SG and HD shared haplotypes lineages and were polyphyletic. To explain the phylogeny structure, Hoelzel *et al.* (2001) suggested a female founding event for the PV population and no further female immigration to the colony.

Individuals from Sea Lion Island (SLI – Falklands) and from Elephant Island (EI - South Shetlands) had never been analysed before. They are both part of the South Georgia stock and could provide important evidence on the genetic differentiation pattern present in the stock. In particular SLI, with its geographically intermediate position, could serve as a conduit between the PV and the SG colonies and hence would be expected to show intermediate differentiation between the two colonies.

Long-distance excursions have been documented for individual of both sexes and for different populations. For foraging trips, females tend to travel further, while males often forage near the breeding colonies (McConnell and Fedak 1996; Campagna *et al.* 1998). A juvenile seal marked on MQ was resighted moulting on Peter 1 ∅Y (approximately 5200 km east of MQ) and again on MQ few months later (Hindell and McMahon 2000). This illustrates that southern elephant seals have the potential to disperse and to utilise all regions of the southern oceans. Subadult males from PV have been resighted on Sea Lion Island during the moulting season. Movements of SLI seals during moulting periods have also been recorded between SLI and both Livingston Island and King George Island, two islands that are part of the South Shetland Archipelago some 1200 km away from SLI. A young male from SLI has been also resighted on the further distant Gough Island, more than 3000 km from SLI (Galimberti unpublished data). The resighted animals were mostly males tagged on SLI as non-reproductive males, apart from two adult females. The two females bred on SLI and were resighted after about two months from their departure on different islands. The first one was seen on Livingston Is. and the second one on King George Island.

Both females and males from Sea Lion Island showed a high level of site fidelity, with females being more site-faithful than males (Fabiani *et al.* in prep.). However, during the mark-recapture study that has been carried out on SLI since 1995, one female from SLI has been resighted at PV. The female came ashore for breeding on SLI the first year, in 1995, she skipped one year and she was seen again in 1997. She has not been seen again on SLI after 1997, but she was resighted breeding at PV in 2000. This would be the first case of an adult female southern elephant seal using two different colonies as breeding sites.

V.1.2 Aims

In this chapter, individuals from SLI and EI were analysed at both microsatellite and mtDNA markers. Then, the results from these two colonies were compared with data from other colonies of southern elephant seals, to:

a) quantify the genetic variation in two populations not previously studied (EI and SLI);

b) assess the pattern of genetic variation and differentiation among the putative populations of the Southern Ocean;

c) assess the pattern of dispersal of males and females and possible differences between them.

V.2 RESULTS

MICROSATELLITE LOCI

V.2.1 Microsatellite genetic variation

Each pair of loci in each colony (SLI, EI, SG, PV) was tested for linkage disequilibrium. No test was significant in any population, therefore independent segregation of alleles at the different loci was assumed in subsequent analyses. Hardy-Weinberg equilibrium was tested for each population at each locus, and no significant deviation was found ($P > 0.1$ for each test).

V.2.1.1 Geographic variation of microsatellite allelic frequencies

All seven loci showed similar levels of variation among populations. Observed heterozygosity values ranged from 0.56 to 0.91. The most variable locus was M2b with ten alleles and the least variable was Hg4.2 with four alleles. The total number of alleles scored in each population was highest in SLI (39 alleles) and lowest in PV (32), most probably due to the larger number of individuals analysed at Sea Lion. Values of allele richness and polymorphism for each population and locus are showed in Table V.1. F_{IS} values were low for each marker, both within (from -0.178 to 0.112) and among populations (from -0.055 for SG to 0.008 for SLI) and none of them was statistically significant ($P > 0.1$ always). Considering only the five markers screened for the four populations, private alleles (i.e. alleles whose occurrence is restricted to only one population) constituted 10% of all alleles. Four private alleles were observed in the SLI population and one in the SG population. One more private allele was present in SLI at the marker PV9. All private alleles occurred at very low frequencies ($P \leq 0.033$) (Table V.2).

In Table V.3 the allele frequencies at the BETA loci in the SLI and EI populations were compared with published data for HD, MQ (Slade *et al.* 1998), PV and SG (Hoelzel *et al.* 2001). At these loci, the PV population was differentiated from HD ($G = 27.9$, $df = 8$, $P = 0.0005$), MQ ($G = 46.23$, $df = 8$, $P < 0.0001$), EI ($G = 30.3$, $df = 10$, $P = 0.0008$) and SLI ($G = 33.63$, $df = 10$, $P = 0.0002$). The population of SG was differentiated from MQ ($G = 26.72$, $df = 9$, $P = 0.0016$) but not significantly from the other populations ($P > 0.1$). SLI differentiated from MQ ($G = 45.8$, $df = 8$, $P < 0.0001$) and HD ($G = 25.14$, $df = 9$, $P = 0.0028$), but not from

Locus		EI	SLI	PV	SG
Hg4.2	n individuals	46	263		
	n alleles (all. richn.)	4 (3.97)	4 (3.98)		
	F_{IS}	0.029	-0.015		
	H_O	0.5652	0.5817		
	H_E	0.5819	0.5729		
Hg6.3	n individuals	46	263	24	30
	n alleles (all. richn.)	5 (4.71)	7 (6.55)	4 (4.29)	7 (4.76)
	F_{IS}	0.093	0.079	-0.178	0.112
	H_O	0.5652	0.5741	0.6667	0.5667
	H_E	0.6228	0.6236	0.5564	0.6261
Hg8.10	n individuals	46	263	36	36
	n alleles (all. richn.)	6 (5.88)	6 (5.66)	6 (5.41)	6 (5.88)
	F_{IS}	-0.051	-0.038	0.064	-0.111
	H_O	0.7826	0.7833	0.6944	0.8333
	H_E	0.7368	0.7533	0.7414	0.7512
Hg8.9	n individuals	46	261	33	36
	n alleles (all. richn.)	8 (7.75)	9 (6.22)	7 (7.24)	7 (7.49)
	F_{IS}	0.009	0.034	-0.005	-0.077
	H_O	0.7174	0.6782	0.5455	0.6667
	H_E	0.7241	0.7022	0.5344	0.6111
M11A	n individuals	46	263	24	31
	n alleles (all. richn.)	7 (5.25)	7 (5.00)	6 (5.76)	5 (5.36)
	F_{IS}	-0.164	-0.013	-0.023	-0.097
	H_O	0.9130	0.7795	0.7500	0.8710
	H_E	0.7857	0.7695	0.7179	0.7825
M2b	n individuals	46	263	79	40
	n alleles (all. richn.)	9 (8.11)	10 (8.50)	9 (8.45)	9 (8.51)
	F_{IS}	0.060	-0.010	0.104	-0.078
	H_O	0.8043	0.7338	0.7342	0.8750
	H_E	0.8552	0.7265	0.8139	0.8022
Pv9	n individuals	46	261		
	n alleles (all. richn.)	4	5		
	F_{IS}	-0.082	-0.032		
	H_O	0.6087	0.4636		
	H_E	0.5631	0.4494		

Table V.1 Polymorphism at each locus for all sampled populations: n allele (all. richn.) = total number of alleles and allele richness; F_{IS} = Wright's inbreeding coefficient; H_O = observed heterozigosity; H_E = expected heterozigosity. Allele richness was based on minimum population size of 24 individuals for loci screened in 5 populations (Hg6.3, Hg8.10, Hg8.9, M11a, M2b) and of 46 individuals for loci screened only in SLI and EI (Hg4.2, Pv9)

	Hg4.2			*Hg6.3*			*Hg8.10*			*Hg8.9*			*M11a*			*M2b*			*Pv9*	
	EI (46)	SLI (263)		EI (46)	SLI (263)		EI (46)	SLI (263)		EI (46)	SLI (261)		EI (46)	SLI (263)		EI (46)	SLI (263)		EI (46)	SLI (261)
135	0.023	0.036	***215***	0.022	0.034	***173***	0.011	0.057	***178***	0.022	0.044	***141***	0.217	0.220	***234***		0.002	***162***	0.185	0.105
137	0.543	0.586	***216***		0.002	***175***	0.304	0.211	***182***	0.152	0.123	***143***	0.272	0.285	***236***	0.120	0.084	***164***	0.620	0.715
139	0.348	0.268	***217***	0.391	0.432	***177***	0.315	0.373	***183***	0.044	0.023	***145***	0.065	0.046	***238***	0.011	0.006	***166***	0.152	0.172
141	0.087	0.110	***219***	0.467	0.428	***179***	0.250	0.226	***184***	0.478	0.504	***147***	0.152	0.184	***240***	0.120	0.078	***168***		0.002
			221	0.109	0.084	***181***	0.087	0.061	***185***	0.109	0.058	***149***	0.272	0.257	***242***	0.120	0.042	***170***	0.043	0.006
			223	0.011	0.008	***183***	0.033	0.072	***186***	0.109	0.138	***151***	0.022	0.004	***244***	0.250	0.487			
			225		0.013				***187***	0.011	0.040	***153***		0.004	***246***	0.185	0.087			
			231						***189***	0.076	0.061				***248***	0.054	0.072			
									191		0.010				***250***	0.098	0.065			
															252	0.043	0.078			

	PV (24)	SG (30)		PV (36)	SG (36)		PV (33)	SG (36)		PV (24)	SG (31)		PV (79)	SG (40)
215	0.042	0.050	***173***	0.069	0.013	***178***	0.030	0.028	*141*	0.146	0.210	***234***	0.031	0.062
216			***175***	0.083	0.263	***182***	0.182	0.153	*143*	0.125	0.307	***236***	0.044	0.038
217	0.500	0.517	***177***	0.431	0.361	***183***			*145*	0.083	0.129	***238***		
219	0.438	0.317	***179***	0.056	0.222	***184***	0.652	0.583	*147*	0.458	0.177	***240***	0.114	0.013
221	0.021	0.050	***181***	0.222	0.069	***185***	0.030	0.069	*149*	0.167	0.177	***242***	0.070	0.075
223		0.017	***183***	0.139	0.069	***186***	0.076	0.139	*151*	0.021		***244***	0.285	0.338
225		0.017				***187***	0.015	0.014	*153*			***246***	0.095	0.200
231		0.033				***189***	0.015	0.014				***248***	0.051	0.050
						191						***250***	0.044	0.060

Table V.2 Allele frequencies of 7 microsatellite loci for El and SLI and of 5 microsatellite loci for PV and SG. In parenthesis is the sample size for each population and locus. Allele sizes (bold) are shown in bp; underlined alleles represent private alleles

	Populations					
Allele size (bp)	EI	SLI	PV	SG	HD	MQ
269	4	40	3	4	15	23
274	7	13	0	0	3	6
279	10	51	3	7	11	7
284	12	49	7	8	7	6
289	35	230	23	28	15	11
294	50	331	48	35	36	38
299	25	108	22	13	15	14
304	4	18	1	5	7	0
309	1	2	0	3	2	1
314	4	62	10	6	6	4
319	4	34	9	4	6	6
324	2	17	12	1	3	1
329	3	49	4	7	2	1
334	10	44	6	2	0	10
339	1	4	0	1	0	0
n individuals	46	263	37	31	32	32

Table V.3 Allele frequencies at the Beta loci for each population. Data from HD, MQ and part of the data from SG are from Slade et al. (1998); the rest of the data from SG and those from PV are from Hoelzele et al. (2001)

EI ($P = 0.96$). EI was not different at these loci from HD ($P = 0.13$) but it was from the MQ population ($G = 18.47$, $df = 6$, $P = 0.0051$).

V.2.2 Population differentiation

V.2.2.1 Allelic and genotypic distributions

In the analysis of the allelic distribution (null hypothesis: the allelic distribution is identical across populations), the four populations differentiated among each other ($P < 0.008$ for all pairs of populations across all loci – correction for multiple comparisons applied). Only the locus Hg6.3 did not differentiate any of the populations. Two to three loci had significant differences in allele frequencies between PV and the other populations (loci Hg8.10, M2b, M11a; Fisher's exact probabilities, $P \leq 0.00019$). Only one locus (M2b) differentiated SLI, EI and SG among each other.

The test on the distribution of genotypes (null hypothesis: the genotypic distribution is identical across populations) confirmed the differentiation, with M2b, M11a and Hg8.10 being the loci that differentiated the most among the populations ($P < 0.005$). The loci Hg4.2 and Pv9 did not differentiate SLI from EI.

V.2.2.2 Population structure

The proportions of the genetic variation attributable to genetic difference between populations quantified with θ (Weir and Cockerham 1984) and Rho_{ST} (Slatkin 1995) are shown in Table V.4. θ and Rho_{ST} calculated across the four populations were 0.016 (95% CI: 0.005 – 0.028; $P < 0.001$) and 0.0062 (95% CI: 0.0021 – 0.0315; $P = 0.008$), respectively. Rho_{ST} and θ values were consistent in all the comparisons except for SG vs PV and SLI, and for SLI vs EI. Theta (θ) showed significant level of differentiation in all pairwise comparisons except for SG vs EI. On the other hand, Rho_{ST} was significant only for the PV vs EI and PV vs SLI comparisons.

Genetic distances, measured as D_A (Nei 1983), ranged from 0.018, between EI and SLI, to 0.073, between EI and PV. Values of $(\delta\mu)^2$ were consistent with D_A values and ranged from 0.005 (EI vs SLI) to 0.106 (EI vs PV). Details are in Table V.5

	PV	EI	SLI	SG
PV		0.0412 *	0.0348*	0.0247*
EI	0.0440 *		0.0085*	0.0057
SLI	0.0341 *	-0.0035		0.0065*
SG	0.0294	-0.0084	-0.0051	

Table V.4 Genetic differentiation for *Rho* and θ between populations. θ are reported in the upper matrix and Rho_{ST} values in the lower. *, significant after adjustment for multiple comparisons. SLI-EI values were calculated on seven loci

	PV	EI	SLI	SG
PV	-	0.106	0. 081	0.098
EI	0.073	-	0.005	0.011
SLI	0.053	0.018	-	0.010
SG	0.052	0.043	0.032	-

Table V.5 Genetic distances between populations. D_A values are reported in the lower matix and $(\delta\mu)^2$ values are reported in the upper matrix

V.2.2.3 Population assignment test

In the assignment test, when EI and SLI were compared at seven loci, 69% of individuals were classified to the population of origin: 52% for the EI population ($P = 0.036$) and 71% for the SLI population ($P = 0.004$). Percentages of correct classifications for SLI and EI were smaller when the test was carried out with the other populations and on only five loci (Table V.6). For both SLI and EI only 41% of individuals were assigned respectively to the nominal populations. In total, for the four populations, only 42% of elephant seals were correctly classified to the original population. EI had the majority of 'misassignments' (55% of total misassignments) to SLI and, similarly, the majority of missassigned individuals from SLI were allocated to EI (41% of total SLI misassignments). For both populations, the smallest number of misassigned individuals was to PV. SG had only 36% of genotypes correctly classified ($P = 0.10$) and 25% assigned to SLI. PV had the highest proportion of individuals correctly assigned (56%, $P = 0.0004$) and the smallest numbers of misassignments from SLI and EI.

	Assigned to population:					
Nominal pop.	PV	EI	SLI	SG	n individuals	Corr. (%)
PV	19	4	5	6	34	56%
EI	4	19	15	8	46	41%
SLI	37	64	108	54	263	41%
SG	7	7	9	13	36	36%

Table V.6 Assignment of individuals to populations and percentage of correct classification, Corr. (%), based on microsatellite data

MITOCHONDRIAL DNA

V.2.3 MtDNA diversity

The sequences from SLI and EI were compared at the 299bp segment of the control region with published data from SG, PV (after Hoelzel *et al.* 1993a, 2001), MQ and HD (after Slade *et al.* 1998).

Forty-two variable sites defined 67 haplotypes for the 299bp control region genotypes among the six populations. Six sites showed transversion substitutions, while all the others showed transitions. Five transversions occurred in haplotypes of SG, while one transversion occurred among seals sampled on EI. All MQ haplotypes shared two fixed mutations (positions 185, 351). The two mutations were exclusive to the MQ haplotypes with the remarkable exception of one seal sampled on SLI (haplotype *sli_blob*). The genotype *sli_blob* was the only one that did not fit with the other lineages represented at SLI, and it was instead well represented in the MQ lineage. All PV haplotypes also shared two fixed mutations (positions 277, 290), and in this case neither of them was found in any of the other sampled seals.

Nucleotide diversity (π) varied significantly among populations, ranging from 0.3 ± 0.2% (PV) to 3.2 ± 1.6% (SLI). Gene diversity was calculated for only four populations because the MQ and HD samples were too small for the analysis. $\hat{H}$ was highest for SG (0.982 ± 0.018) and lowest for PV (0.685 ± 0.024), while SLI and EI showed a gene diversity value very close to that shown by SG (Table V.7).

Population	n	Poly sites	Haplo	π	$\hat{H}$
SLI	57	29	20	0.032 (0.016)	0.952 (0.009)
EI	30	24	12	0.032 (0.017)	0.959 (0.021)
PV	32	2	3	0.003 (0.002)	0.685 (0.024)
SG	28	25	24	0.028 (0.015)	0.982 (0.018)
HD	6	13	6	0.023 (0.015)	-
MQ	5	11	5	0.019 (0.012)	-

Table V.7 Genetic variability based on mtDNA analysis (299 bp). For each population, number of individuals (n), number of polymorphic sites (Poly sites), number of haplotypes (Haplo), nucleotide diversity (π) and gene diversity ($\hat{H}$) are shown.

Results from the mismatch distribution tests were not significant, as the differences between pairs of haplotypes in each population shown multimodal distributions (0.01 < *raggedness index* < 0.1; $P > 0.09$ for each test). The test was not run for MQ and HD. Fu's test for neutrality was not significant for SLI, EI and PV ($P > 0.05$ – no evidence of selection acting) but provided a significant and large negative value of *Fs* for SG that might indicate a demographic expansion of the population ($P < 0.0001$). However, Tajima'*D* test did not confirm the result for SG ($D = -0.09$, $P > 0.1$) and provided no evidence of selection acting on this part of the mtDNA control region in any population ($P > 0.05$ for each population).

V.2.4 Differentiation and distribution of 299bp mtDNA genotypes

There was very weak but significant differentiation between SLI and EI (ϕ_{ST} = 0.049). Differentiation was not significant between HD and either SLI or EI (Table V.8). Both Sea Lion and Elephant Islands' populations were instead differentiated from the closer SG, with ϕ_{ST} values of 0.149 and 0.116, respectively. High and similar differentiation was present between the two populations and MQ (0.455 for SLI and 0.444 for EI). Both SLI and EI were most differentiated from PV, with which they had no haplotypes in common.

	SLI	EI	PV	SG	HD	MQ
SLI	-	22 (0)	2 (3)	18 (0)	11 (0)	7 (0)
EI	0.049	-	2 (3)	19 (0)	11 (0)	5 (2)
PV	0.526*	0.625*	-	1 (3)	2 (3)	0 (12)
SG	0.149*	0.116*	0.582*	-	9 (0)	3 (2)
HD	0.069	0.082	0.811*	0.222*	-	3 (3)
MQ	0.455*	0.444*	0.918*	0.522*	0.555*	-

Table V.8 Differentiation among populations for mtDNA. ϕ_{ST} values are shown below the diagonal; *, significant after adjustment for multiple comparisons. Shared mutations and number of fixed differences (in brackets) are shown above the diagonal

The number of shared mutation ranged from 0 (between MQ and PV) to 22 (between SLI and EI). PV showed the smallest number of shared mutations (maximum 2) and the highest number of fixed differences (12 from MQ). SLI did not show any fixed difference when compared with all but PV and reported a maximum of 22 shared mutations with EI. EI had a similar pattern, except for showing two fixed differences from the MQ population. Details are in Table V.8.

V.2.5 Genetic distance and relationship between populations

Nucleotide divergence between populations (D_a) was highest between MQ and PV (0.043) and lowest between SLI and EI (0.001). SLI showed similar divergence from SG and HD (0.004 and 0.002 respectively) despite the geographical distance separating SLI from HD being much greater than that between SLI and SG. Similarly, the divergence value between SLI and PV (0.018) was very close to the divergence between the SLI population and the very distant MQ (0.019). The same pattern was also seen for the EI population (Table V.9)

	SLI	EI	PV	SG	HD	MQ
SLI	-	0.001	0.018	0.004	0.002	0.019
EI	0.026	-	0.021	0.002	0.003	0.018
PV	0.032	0.035	-	0.017	0.020	0.043
SG	0.027	0.026	0.029	-	0.006	0.022
HD	0.024	0.024	0.031	0.027	-	0.020
MQ	0.040	0.040	0.052	0.041	0.038	-

Table V.9 Nei's D_{xy} (below the diagonal) and D_a (above the diagonal)

Out of the 67 haplotypes defined at this part of the mtDNA control region, nine where shared between two populations. Only one was found in three populations and it was the most commonly recorded haplotype (*sli_probo*), present in SLI, EI and SG in more than one copy (Fig V.2). The shared haplotypes were always among SLI, EI and SG with the remarkable exception of one that was shared between one seal of SLI and one seal from the very distant MQ (haplotype *sli_blob*). The populations that had the most genotypes in common were SLI and EI (eight). No shared genotypes were found between PV and any of the other populations and the same was true for HD (but in this case the sample size was much smaller). The overall frequencies and relationships among the 67 unique sequences are represented with the network in Fig V.3. The haplotypes from particular populations tended to occupy specific portions of the network. Left and right extremes were respectively occupied by the MQ and PV genotypes; four haplotypes from HD clustered together while two (*hd2* and *hd6*) were located in another part of the network. Genotypes from SLI, EI and SG were located in the centre of the network and the most common haplotype

	1111122222	2222222222	2222222223	3333333333	33						
	3356877779	8899000256	6278999770	0011122344	55						
	2462534579	4502036861	2334915785	7901229029	19			POPULATIONS			
consensus	CCGTTTATGG	ATTCTAAATG	AATGCACACC	TGGGGCGACA	CG	SLI	EI	SG	HD	MQ	AR
sli blob	T...**C**.....	.C.TC.....	........T.	.......G..	**T**A	1				1	
sli bo	.........A	....C.....	...A......	...A......	..	4					
sli che	.....CG..A	....C.....	...A......	...A......	..	2	2				
sli zeta	.....C...A	....	...AT.....	....	..	1					
sli_fat	.....C....	.........A	...AT...T.	...A...G..	..	3					
sli fina	T.........	.C........	....	.A........	..	4	2				
sli gita	....	.C.......A	.........T	...A...G..	..	2					
sli glu	T.........	.C......CA	....	.A........	..	1	1				
sli ielo	.T........	.........A	....	C.AA......	..	1					
sli_leo	.....CG..A	....	...AT.....	....	..	6					
sli ovo	.....CG..A	.C..C.....	...A......	...A......	..	3	2				
sli oz	....	G.........	...AT..GT.	...A.T.G..	..	4					
sli probo	....	GC........	.........T	.......G..	..	3	5	4			
sli sal	.....C....	.........A	...AT...T.	.......G..	..	5		2			
sli scar	T.........	.C........	..C.......	.A........	..	1					
sli sebi	.....CG..A	....C....A	.G.A......	...A......	..	2	1				
sli sil	.....CG..A	G...C.....	...A......	...A......	..	3	1				
sli tom	T.........	G........A	G...T.....	.A........	..	3	3				
sli uga	.....CGC.A	....	...AT.....	....	..	2					
sli_uno	T.........	.C........	..C.......	.A.....G..	..	6					
ei01	T.........	GC........	....	.A........	..		1				
ei05	....A.....	.........A	....	....	.A		1				
ei08	.........A	.........A	.........T	C.A.......	..		2				
ei10	.....C....	GC........	.........T	.......G..	..		2				
ei11	.....CG..A	....C.....	...A......	...A......	.A		1				
ei15	....	GC........	........TT	.......G..	..		1				
ei20	....	GC........	.........T	C......G..	..		1				
ei21	....A.....	.......G.A	....	.A........	.A		1				
ei22	.....CG..A	....C.....	....	...A......	..		1				
ei23	.....C....	.C.......A	.G.......T	...A......	..		1				
ei27	T......C..	........CA	....	.A........	..		1				
sg01	......G...	GC...TT...	.........T	.......G..	..			1			
sg03	.....CG..A	....C.....	....	...A..AG..	..			1			
sg04	....	.C.......T	.........T	...A...G..	..			1			
sg05	.....CG..A	.C..C.....	...A..T...	......AG..	..			1			
sg06	....	.......G.A	....	.A....AG..	..			1			
sg07	.....CG..A	.C..C.T...	...A......	...A..AG..	..			1			
sg08	......G...	....	....	...A..AG..	..			1			
sg09	T.........	G........A	G...T.....	.A....AG..	..			1			
sg10	.....CG..A	G.........	...A......	...A..AG..	..			1			
sg11	......G...	.G........	.........T	...A..AG..	..			1			
sg12	T.........	GC........	....	.A.....G..	..			1			
sg13	......G...	GC........	.........T	.......G..	..			1			
sg14	....	.C........	....	.A....AG..	..			1			
sg15	T.........	.C.......A	....	.A....AG..	..			1			
sg16	T.........	.C........	....	.A....AG..	..			1			
sg17	.A....G...	.C...T....	...AT....T	....A..G..	..			1			
sg18	T.........	.C......CA	....	.A....AG..	..			1			
sg19	.........A	.........A	.........T	..A...AG..	..			1			
sg20	.....C....	.C.......A	.G.......T	...A..AG..	..			1			
sg21	.....CG..A	....C..G..	...A......	...A..AG..	..			1			
sg23	....	GC........	.........T	......AG..	..			1			
sg24	....	.C........	....	......AG..	..			1			
hd1	.....C....	.C........	...A......	....	..				1		
hd2	T.........	.C.......A	....T.....	.A......T.	..				1		
hd3	..A..C.C..	GC........	...A......	...A......	..				1		
hd4	.....C....	.C........	...A......	...A......	..				1		
hd5	..A..C.C..	GC........	...A......	....	..				1		
hd6	T.........	.C..C.....	...A......	.A.....G..	..				1		
mq2	TT.C**C**.....	.C.TC.....	........T.	....	**T**.					1	
mq3	T...**C**.....	.C.TC.....	........T.	....	**T**.					1	
mq4	TT.C**C**.....	G..TC.....	G....G..T.	........TG	**T**.					1	
mq5	T...**C**.....	.C..C.....	........T.	....	**T**.					1	
pv_a	........**A**.	G.**C**.......	...AT.....	......AG..	..						10
pv_b	.....C..**A**.	G.**C**.......	...AT.....	......AG..	..						12
pv_c	.......C**A**.	G.**C**.......	...AT.....	......AG..	..						10

Fig V.2 Polymorphic sites observed in the 299bp segment of the mtDNA control region in six populations. Vertical numbers indicate the position relative to sequences from Hoelzel *et al.* (1999); in bold type are the fixed mutations within population; the transversions are underlined. The consensus sequence (*consensus*) has been used as reference sequence. On the right, haplotype frequencies are given for each putative population and shared sequences are indicated (dotted outlines)

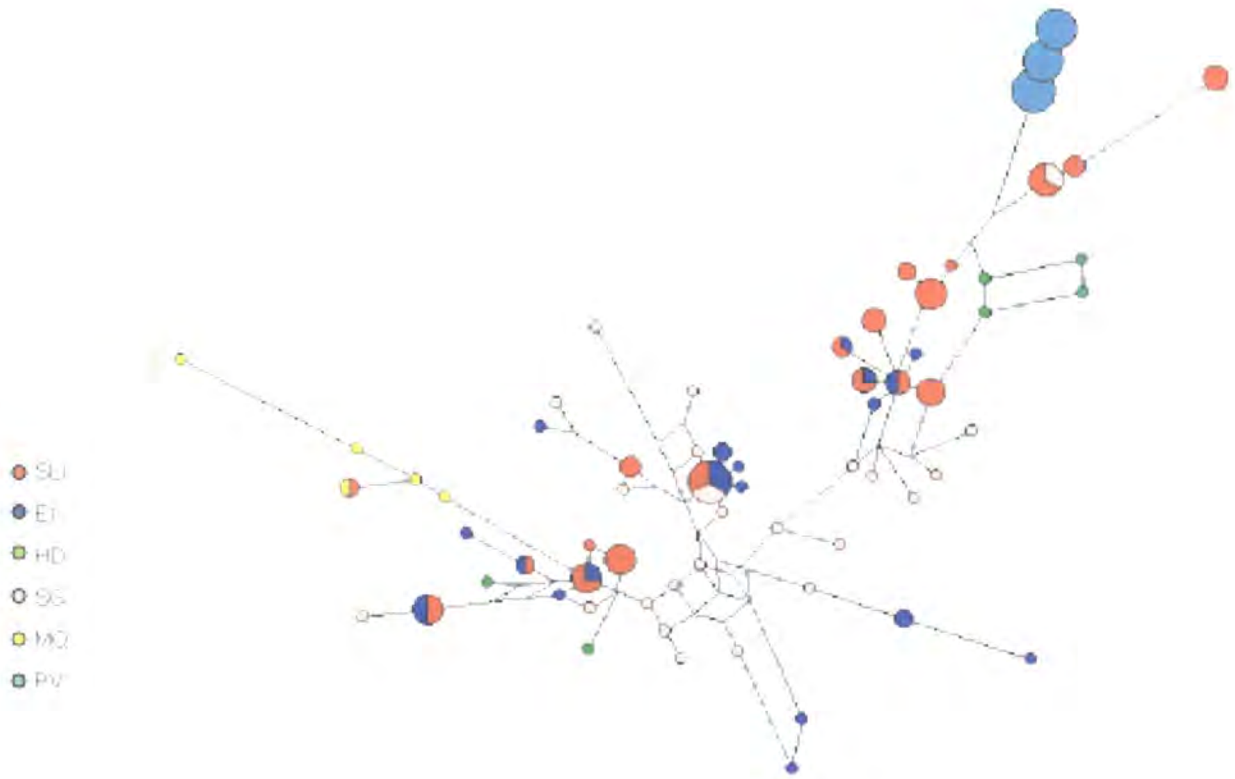

Fig V.3 Median joining network of the 67 mtDNA sequences (299 bp) from the six populations. The size of the nodes indicates the overall frequency of the genotype. Nodes without a corresponding haplotype indicate hypotetical intermediate genotypes. The link length is proportional to the number of mutations between two nodes. Each population is indicated with a different colour (left) and haplotyopes shared between two or more populations are represented in multiple colours was the centre of a star-like structure, linked with rarer haplotypes belonging to SG and EI. Two SG genotypes were shared with SLI, both in multiple copies, while the remaining seals from SG had unique haplotypes. The majority of them were linked among each other and few to genotypes from EI and SLI. Only three seals from SLI and nine from EI had unique haplotypes and SLI unique genotypes were concentrated to the central-right part of the network.

MQ haplotypes were well separated from the central network, through a SLI - EI haplotype (separated by 5 mutations). However, one genotype of the MQ group was shared with a male sampled on SLI. Also the Argentinean group was well apart (6 mutations from the closest genotype, *sli_zeta*), with only three haplotypes equally represented among 32 individuals. They were located on a single matrilineage where the two external haplotypes could be derived from the third (*ar1*, in central position) by a single bp mutation.

Phylogenies derived using the neighbour-joining and the maximum parsimony methods showed equivalent topology. The maximum parsimony consensus tree of the 299bp control region is shown in Fig V.4. The tree showed a general 'comb-structure'. However, three main lineages were recognised with bootstrap supports of 90%, 86% and 80%, respectively. The first one included all five haplotypes from MQ, one of which was the same as an individual haplotype from SLI (*sli_blob*); the second lineage included the three PV genotypes, and the third one was represented by two out of the six HD haplotypes (*hd3* and *hd5*). The other four HD sequences were positioned along the main structure. Pairs of haplotypes were also separated from the main structure though not strongly supported (bootstrap supports from 57% to 67%). All but one were pairs of multiple copies haplotypes and mixed populations. Only one pair was represented by unique haplotypes belonging to only one population (*ei05* and *ei21*) and it was the pair more strongly supported (67%). All the remaining haplotypes were distributed along the main structure of the tree.

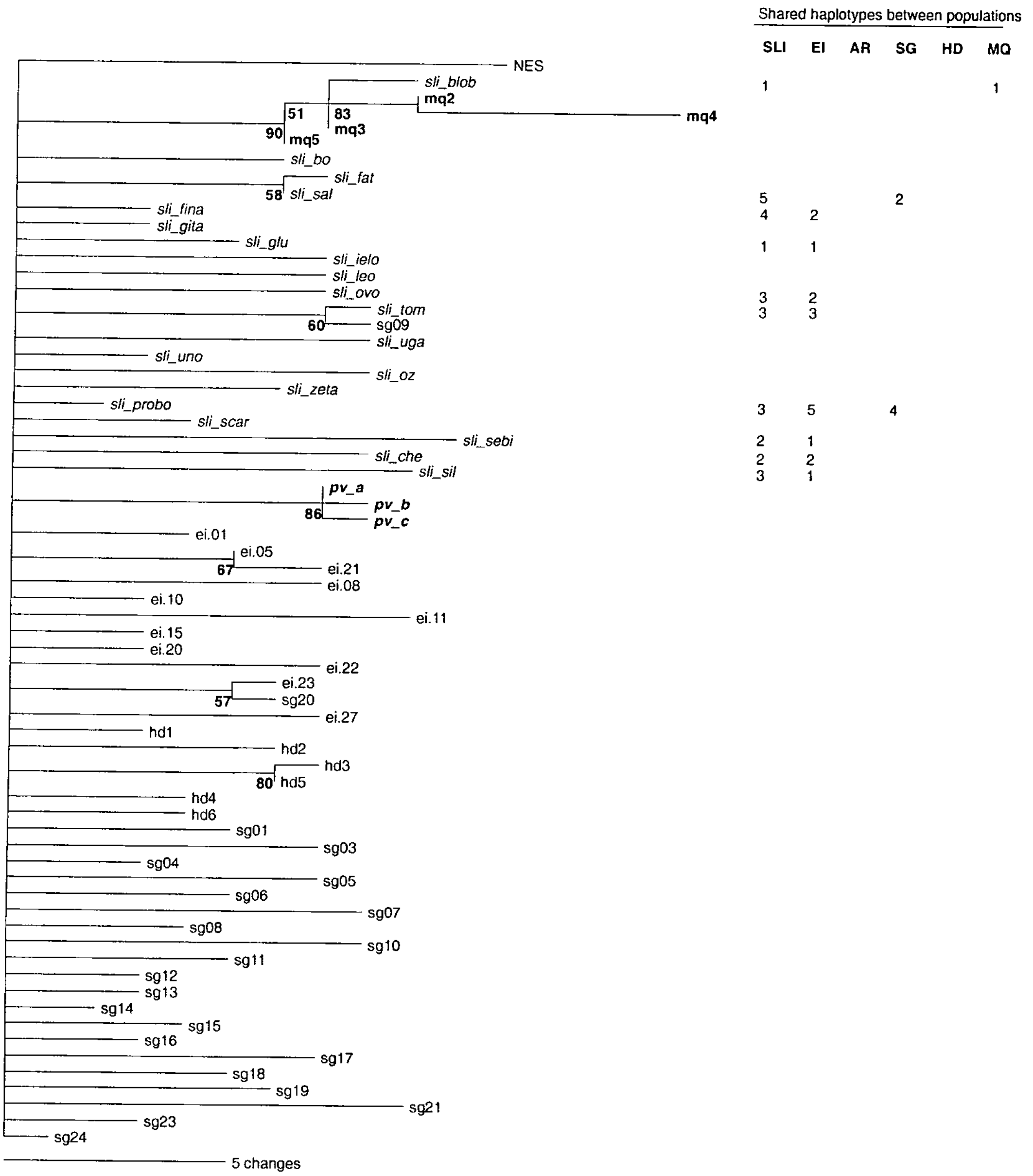

Fig V.4 Maximum parsimony tree of 299 bp of mtDNA control region from the six breeding colonies. Bootstrap supports from 1000 replicates (bold) are given. One northern elephant seal sequence was used as outgroup (nes). On the right, the frequency and the geographical distribution of sequences found in more than one population are shown. SLI haplotypes are indicated in italic while monophyletic groups are indicated in bold type

V.3 DISCUSSION

In this chapter, the genetic variation of nuclear and mitochondrial loci in the southern elephant seal populations of Sea Lion and Elephant Islands was assessed. The pattern of variation was investigated in the context of previous results on breeding colonies belonging to the same South Georgia stock and to the more distant Heard and Macquarie Islands.

V.3.1 Level of genetic variation

The levels of genetic variation detected in the populations of SLI and EI at both microsatellite and mitochondrial markers were high and comparable with the variation present in most other breeding colonies. Nucleotide diversity in the two populations had the same value of 3.2%, which is among the highest reported for a mammal species (see Table 2 in Slade 1997). It was close to those reported for SG, HD and MQ, and an order of magnitude higher than for the PV population (Hoelzel *et al.* 2001). The numbers of haplotyopes found at the breeding colonies of SLI and EI were also elevated, respectively 20 (out of 57 seals) with 29 polymorphic sites, and 12 (out of 30 seals) with 24 polymorphic sites. Both values were similar to those reported for SG and much greater than the number of haplotypes and mutations screened for the geographically close PV colony. It thus seems to confirm that PV might have had a different history compared to the other populations, and that there is a very low gene flow between the Argentinean population and the nearby breeding sites.

V.3.2 Genetic differentiation and population structure

V.3.2.1 Population structure within the South Georgia stock

In previous studies (Hoelzel *et al.* 1993a; Slade 1997; Slade *et al.* 1998; Hoelzel *et al.* 2001), striking differences were found between SG and PV, similar to differences found between SG and the very distant MQ. However, no data were available from any of the other colonies belonging to the same South Georgia stock. In this study, equivalent genetic divergence was found between PV and all the island sites of the stock.

Microsatellite data showed similar variation among the populations of SLI, EI, SG and PV and accounted for a between-population difference of 1.6%. However, most of this variation was attributable to the genetic differences between PV and the other colonies. θ values between PV and SLI (3.5%) and PV and EI (4.1%) were greater than those observed between the peninsular colony and SG (2.5%). This result is surprising, since SLI is geographically intermediate between PV and SG and it was hypothesised as a conduit for dispersal between SG and the Argentinean population (Hoelzel *et al.* 2001).

The extent of genetic differentiation of PV from SLI and EI was also high at the mitochondrial locus. For the two islands, 53% and 63% of the genetic variance was due to differences with PV, values very similar to that between SG and PV (58%). The presence of exclusive haplotypes at PV (Hoelzel *et al.* 1993a) was also confirmed, since no genotypes were shared between the Argentinean colony and the two islands. In a median joining network, the three PV haplotypes were not only on a separate branch, but they were also at the very extreme of the network. Networks of this kind frequently show common haplotypes in more central positions, linked with more rare and external haplotypes (O'Corry-Crowe *et al.* 1997; Bandelt *et al.* 1999; Girman *et al.* 2001). In this network, however, the PV genotypes followed a different pattern: they all showed elevated frequencies and the two external ones could be derived from the central one by only a single bp mutation. Hoelzel *et al.* (2001) suggested a single founding event for PV with no further significant female recruitment. The elevated differentiation between PV and SLI and EI, data from genetic distance and divergence, and the absence of shared haplotypes between PV and the two islands seem to support this evidence. The genetic distance between two populations, in fact, gives a relative estimate of the time that has passed since the two populations were a single cohesive unit. Small estimations of distance may indicate population substructure (i.e. subpopulations in which there is random mating, but between which there is a reduced amount of gene flow). However small values of distance may also be present because the populations are completely isolated but have only been separated for a short period of time.

High genetic similarity was instead found between SLI and EI, as geographic structure was very weak at both microsatellite and mitochondrial loci. Genetic similarity was also present between these two colonies and SG, but in this case the results showed discordance between the two marker sets. In fact, while the mtDNA data indicated significant genetic subdivision between SG and SLI and between SG and EI, results from microsatellite analysis showed very weak subdivision. These results suggest limited female dispersal between SG and the other two colonies and a more pronounced male-biased dispersal among the three colonies.

Weak or no differentiation between SLI and EI, at both microsatellite and mitochondrial markers, suggests either a similar pattern of dispersal between sexes or a relatively short separation time between the two colonies (Slade 1997). Elephant Island was under ice until the last ice-age (about 18 000 years ago) so it is plausible that, becoming available as a breeding site, seals from the Falklands moved south towards it. The two islands are about 1000 km apart, a distance well within the known dispersal capacity of the species (McConnell and Fedak 1996; Hindell and McMahon 2000). Data on the colony of Sea Lion Island indicate that the population size has remained almost constant since 1989 (Galimberti *et al.* 2001), although it could represent what is left from a formerly larger population that used to occupy other sites in the rest of the Falklands (Galimberti and Boitani 1999). This could support the hypothesis of movements towards new breeding sites. Moreover, resightings of seals from SLI have been reported on other islands of the South Shetland Archipelago, on King George Island and Livingston Island, both about 1100-1200 km away from SLI (Galimberti unpublished data). The majority of them were 2-3 year old males, while one was a female previously observed breeding on SLI for three years (she returned to breed on SLI after being on Livingston Island). The hypothesis of movements of seals between SLI and EI is also supported by the data on genetic distances. The genetic distance (D_A) between the two islands (1.8%) was smaller than the distances between SG and either EI (4.3%) or SLI (3.2%). SG might have been isolated from the two islands by the presence of ice. The ice might have receded from EI and initially facilitated the breeding dispersal between the South Shetlands and the Falklands, leaving the SG colony relatively isolated. However, no evident factors seem to explain why patterns of dispersal were excluding the colony of SG.

Similar distribution of genetic variation among SG, SLI and EI at microsatellite loci was also confirmed by the population assignment test. This test was carried out to assess whether there was sufficient differences between populations to make an individual's genotype characteristic of the site where it was sampled. With this test, only about 40% of the individuals from each population were assigned to the colony where they were sampled. However, assignments were significantly more succesful between SLI and EI when seven markers instead of five were considered.

V.3.3 Population structure among stocks

When MQ and HD were included in the analysis, the extent of genetic differentiation between colonies was not always related to the geographical distances between them. Despite the geographical distances separating them, the mtDNA data indicated greater similarity between SLI (or EI) and HD than between SLI (or EI) and SG, where the geographical separation between populations is much smaller. Genetic divergence between SLI and HD was the same as that estimated between SLI and SG, but the geographical distances between the colonies are very different. SLI is 1500 km from SG, while it is some 7600-7700 km from HD. A similar genetic pattern (and similar distance) was also shown by EI, which was as genetically distant from SG as from HD (and geographically much more distant from the second than from the first). Moreover, the levels of genetic structure between SLI and HD and between EI and HD were not significant while they were significant between each of the two islands and SG (ϕ_{ST} of 15% between SLI and SG, and of 12% between EI and SG). However, the situation was different when the BETA loci were compared. In this case, with data from a larger HD sample, the two loci were not able to differentiate among the four colonies (SLI, EI, SG and HD).

Even though none of the HD mitochondrial genotypes were found in any of the other populations, the data show higher genetic similarity between this island and the South Georgia stock than previously thought (Slade *et al.* 1998). In the median joining network, four of the six HD haplotypes were grouped together and connected to the same branch as the PV haplotypes. The other two HD haplotypes, however, were positioned separately at the other end of the network, among haplotypes belonging to SLI, SG and EI. The number of haplotypes available (and their frequencies) from HD was very small, hence results should be interpreted with caution. However, as the HD group was not well separated from the rest, it is plausible that other haplotypes from and increased sample from the same colony would occupy intermediate positions between these four and the other two.

The phylogenetic tree confirmed the pattern of genotype distribution. In previous studies (Slade 1997; Hoelzel *et al.* 2001), the group of four genotypes clustered together into a lineage that included haplotypes *sg22* and was closest to PV. However, with the addition of data from SLI and EI, the topography of the phylogeny changed. Only two genotypes from HD clustered together but they were as distant from the PV lineage as the other sequences of the tree. The other HD genotypes were positioned along the main structure with haplotypes belonging to the other colonies of the South Georgia stock.

A weak genetic structure between HD and the group of SG-SLI-EI is surprising if the physical distance separating these colonies and HD is considered (HD is about 6500 km from SG and 7500 from SLI). However, other colonies in the Kuerguelen stock (Prince Edward Islands, Crozet, Marion, Kerguelen) lie between SG and HD, sometimes less than 1000 km distant from each other. The distance is well within the dispersal capacity of the seals and it is possible that they are used by the seals to move between more distant sites, though no evidence has been found to support this hypothesis. Since genetic similarity between SG and HD had already been detected (Slade 1997; Slade *et al.* 1998), the weak genetic structure between HD and the other two colonies of the stock was not unexpected. Slade (1997) suggested a common origin for the HD and island populations of the South Georgia stock, with a separation during the last glacial maximum (18000 years ago). He proposed that a founding colony could have existed off the coast of southern Africa. Hoelzel *et al.* (2001) suggested instead that most islands might have remained suitable for breeding throughout that Ice Age, or that seals temporarily relocated to the more northern islands (Falklands, Kerguelen, Aukland Islands).

Another discordance between genetic and geographic distances was found between SLI, PV and MQ. The subdivision between SLI and PV was greater than that between SLI and the extremely distant MQ (ϕ_{ST} of 53% and 46%, respectively).

This underlines the high degree of genetic structure within the South Georgia stock. The MQ sample showed extreme differentiation from all other colonies. The pattern was confirmed also for the populations of SLI and EI, at both mitochondrial and BETA loci. Nucleotide divergence between SLI and MQ was moderately elevated (0.019). However, one seal from SLI showed the same haplotype as one individual belonging to the MQ group (haplotype *sli_blob*) (Fabiani *et al.* in press). This exception is extremely remarkable, since MQ genotypes clustered in a strongly supported lineage, separated from the other seals and defined by two fixed differences. No haplotypes from MQ have previously been found in any other colony, and SLI does not share the lineage of MQ with any other seal than *BLOB*. Out of 154 sampled individuals from five different colonies only this seal had the same haplotype as an individual sampled on MQ.

V.3.4 Southern elephant seal dispersal and its implications

The seal from SLI showing the 'MQ haplotype' was an adult male. It is very likely that he was born on MQ and that he covered the distance, some 8000-8500 km from SLI. This is the greatest distance that an elephant seal has been recorded navigating (see Stevick *et al.* 2002 for a review). Because males do not contribute their mitochondrial genotype to the offspring, the haplotype carried by a male can only be sampled during the animal's lifetime and only in the location at which he is present at the moment of sampling. If the male migrates to a different population, so will his haplotype. If it was his mother that migrated to a different population, she would have passed her haplotype onto her offspring and hence more copies would have been available for sampling.

The hypothesis of female elephant seals being philopatric is supported by long-term studies of identified individuals (Lewis *et al.* 1996) although some degree of female dispersal and transfer between colonies may occur. Both females and males on SLI show site fidelity (Fabiani *et al.* in prep.), although males exhibit a more flexible strategy in choosing the sites for breeding than females. Nevertheless, a single female observed breeding on SLI for two years has been resighted breeding on PV two years after her last record on SLI (Galimberti unpublished data). However, the resighting effort on the two colonies is not constant all year around. The population of SLI is small and very well monitored during the breeding season (and at the beginning of moulting), but constant monitoring is not carried out during other periods. On the other hand, the PV colony is much bigger (ca 160 km – Baldi *et al.* 1996) and much more dispersed. The majority of the beaches used by the seals to breed are not under regular observation both during and out of the breeding season, and it is therefore possible that some of the seal movement has been missed.

Nicholls (1970) reported that 60% of branded adult males and 77% of adult females on MQ were found breeding within 4 km of their birthsites. Nevertheless, he noted that males might show higher dispersal with age. Hindell and Little (1988) reinforced the idea of strong female philopatry, recording two 23 yr old females breeding within 1 km of their birthsite on MQ. Southern elephant seals have been recorded travelling up to 3000 km from breeding and moulting sites to remote foraging areas (see Table 7, in Slade 1997). Telemetry data have indicated long-distance excursions for females and more restricted migrations for males (McConnell and Fedak 1996; Campagna *et al.* 1999). Mark-recapture studies on the other hand, have reported resightings of males between different colonies and, more frequently, of young non-breeding males that come ashore for moulting. These data suggest that males, as in the majority of mammals (Dobson 1982), disperse more than females, even if females may forage over greater distance and deeper water than males. The male that migrated from MQ to SLI was present on SLI for two breeding seasons (1995 and 1996). He gained the control of a harem only in 1996 and successfully reproduced, fathering at least 18 offspring. The migration of this male and his elevated reproductive success is an example of the potential homogenising effect that dispersal and mating system can have on the genetic structure of a population.

VI. Discussion

This study demonstrated that southern elephant seals on Sea Lion Island are extremely polygynous, with only a few males fathering a large number of offspring and the majority of males not reproducing at all. Female gregariousness partly accounts for this despotic mating system. Their mating behaviour, ecological features, and male capability of herding and keeping the control of the females, are among the major factors in shaping the mating system of this species. If female gregariousness creates the conditions for polygyny to develop, individual male performance can realise the highest levels of reproductive variance.

High reproductive variance and site fidelity are common aspects in pinniped mating systems. High reproductive variance can strongly affect the level of genetic variation in a population and, at the same time, social structuring can lead to a non-random pattern of genetic relatedness among individuals and to levels of kinship that are higher within than between groups. In addition, female site fidelity and philopatry can contribute to a high gene correlation among offspring and adults within social groups (Chesser 1991a). Only recently have studies been conducted on the distribution of genetic relatedness within pinniped colonies (i. e. in harbour seals: Schaeff *et al.* 1999; grey seals: Pomeroy *et al.* 2000). However, prior to this study, the distribution of genetic relatedness in a highly polygynous pinniped such as the elephant seal had never been investigated, although female site fidelity had been documented (Nicholls 1970; Hindell and Little 1998).

This research has demonstrated that elephant seals have a high genetic diversity (i.e. H_E from 0.57 to 0.77; nucleotide diversity of 3.2%, among the highest reported for mammal species), in spite of the fact that N_e will be reduced in local populations by high male reproductive variance. Moreover, social organisation and site fidelity of females did not lead to any genetic significant sub-structure within the SLI colony. These results suggest high rates of genetic dispersal among colonies and a high overall N_e for the species.

Although both males and females on SLI show a certain degree of site fidelity (Fabiani *et al.* in prep.), individuals do move between colonies. Evidence of gene flow between SLI and other colonies of the stock has been found, although historical and geographical events might have made movements easier between some colonies than others. Despite the extensive mtDNA differentiation, gene flow has also been found between SLI and the very distant Macquarie Island, as the data suggest that a male born on MQ travelled some 8000 kilometres to breed on SLI. His paternal success was high on SLI, and this illustrates the potential homogenising effect that dispersal and mating system can have on the genetic structure of a population.

VI.1 Male reproductive success and behaviour

The level of mating monopolisation was high in the SLI population, confirming previous behavioural results. Moreover, not only were the distributions of observed mating behaviours highly correlated with the distribution of paternity, but also indices based on female holding and copulatory behaviours were very good predictors of individual paternity.

Few studies have investigated the relationship between behavioural and genetic indices of male success in polygynous mammals. In grey seals (Worthington *et al.* 1999), fur seals (Gemmell *et al.* 2001) and Soay sheep (Coltman *et al.* 1999a) behaviour overestimated the variance in male reproductive success and level of polygyny. In Soay rams, behavioural data explained only 22.4% of the variation in the number of offspring sired (Coltman *et al.* 1999a). Instead, in red deer, the distributions of behavioural indices well approximated that of paternity (around 90% of the variance in reproductive success could be predicted from behavioural data) but they did not give an accurate measure of absolute mating success (Pemberton *et al.* 1992). In fact, their behavioural indices, calculated backdating the day of conception from the date of birth, underestimated true paternity for the most successful males and overestimated it for less successful males. They suggested that the discrepancy might arise because the indices were not precise enough: females were not observed every day of the season (and they might move between harems), and many males are simply unsuccessful (even if they can hold a harem for a period of time).

In this study, behavioural indices were strongly correlated with paternity (coefficient of determination, R^2 in the range 0.80-0.99), and predicted individual paternities 60%-100% of the times in each harem. In each harem, the largest proportions of paternities were predicted by identifying the first male seen copulating with the female (73%-100%), information that requires the record of each female's history during the whole breeding season. Lower accuracy, although elevated in the majority of the harems (56-96%), was obtained by knowing the seasonal holder of each harem. For harems where the level of congruence was lower, the correspondence between behavioural and genetic data increased when probable errors in the paternity assignment (harems RUB96, SF96, SI296) were considered, or paternities from other holders (SI296, SI297) were added (see Discussion in Chapter III for details). This result may suggest important practical 'guidelines' for behavioural studies on this colony. It shows that behavioural observations are good predictor of paternity and, consequently, that minimal behavioural observations (i.e. holder males during the peak of the season) can give a good estimate of the distribution of reproductive success among males at SLI.

Quantifying variation in the lifetime mating and paternity success of individuals is essential for determining the potential for sexual selection in a population (Arnold and Wade 1984; Andersson 1994; Coltman *et al.* 1999c). However, very few studies on mammals have measured mating success of individuals of both sexes throughout their lifetime (see Clutton-Brock 1988). Most published paternity studies consider only a few breeding seasons (see Coltman *et al.* 1999c; Worthington *et al.* 1999), even though reproductive success can be very different among years. Social, demographic and ecological factors can change among breeding seasons and hence affect the distribution of seasonal male reproductive success (Coltman *et al.* 1999c). Therefore, seasonal and prolonged collection of data is extremely important to identify patterns of reproductive success among males. The application of genetic markers requires a great deal of effort, as the method requires regular collection of large numbers of samples and long periods of lab work and data analysis. In this context, the possibility of using behavioural estimates collected in short periods of the breeding season to accurately estimate the distribution of paternity would be an important methodological tool for carrying out long-term studies with fewer difficulties.

VI.2 Comparisons of variance in male mating and reproductive success

Currently, only a few comparisons can be made with reported genetic variance of male success, due to both the small number of genetic studies available and the differences in methodological measurements among them. In general, comparisons between studies of variance in reproductive success are difficult, because of differences in methods (e.g. sampling strategy, individuals included in the analysis, calculation of the indices), in spatial and temporal scales or other criteria that may generate bias (Coltman *et al.* 1999c). These differences should be kept in mind when either species or populations are compared.

The opportunity of selection *I* (the ratio of the variance in reproductive success to the square of mean), has frequently been used to compare mating systems between species, mainly because of the easy availability of relevant parameters (mean and variance of reproductive success) for natural populations. However, the method presents many potential drawbacks (Trail 1985; Downhower *et al.* 1987; Kokko *et al.* 1999). As a ratio, it changes when either of its components changes; hence it is dependent of the mean. In particular, mean values less than 1 will have a particularly dramatic effect on *I* (i.e. *I* will increase greatly), thus comparisons of *I* between systems with different mean fitness are questionable (Downhower *et al.* 1987). It is also sensitive to the units of fitness measurement, so *I* values calculated using different fitness units should be compared with caution. Finally, there is no reference value against which the values of *I* can be compared, i.e. *I* does not have a bounded range (Kokko *et al.* 1999).

Mating variance can also vary within species, between and within populations (Petrie and Kempenaers 1998), and among breeding seasons (Coltman *et al.* 1999c; Galimberti *et al.* 2001). Galimberti *et al.* (2002a) used *ENFI* measures (index of fertilisation) to compare male mating success distributions between southern elephant seals of SLI with those of Punta Delgada (DEL). They found that different spatial scales of analysis revealed different magnitudes of variation between populations, and concluded that optimal scales to calculate potential of selection could be different even in different populations of a same species. In this case, different propensities of males to move between zones/areas/harems were observed between the two colonies. This was in part due to the different topographical and geographical characteristics of the colonies. The SLI colony is small, almost isolated from other colonies, and males have very few opportunities to breed away from the island during the same breeding season (Galimberti and Boitani 1999). On the contrary, the population of DEL is not isolated from the other breeding groups of the Peninsula (Campagna and Lewis 1992; Campagna *et al.* 1993) and males have plenty of alternative sites to breed. Keeping in mind these differences, *I* from *ENFI* data was always larger on SLI than on DEL, but the significance of the difference varied depending on the spatial scale of the calculation of *I*. In the present study, the mating variance was confirmed with genetic data that gave an estimate of *I* for the SLI population of 4.04. Moreover, when independent harems were analysed, *I* greatly varied among them (from 2.68 to 11.27) reaching in some harems a value four-fold greater than the smallest value. These results confirm that the level of potential selection can vary locally, and that in elephant seals each harem defines a reproductive context with its level of male competition and polygyny potential.

Although showing different levels of polygyny potential and holder success, each breeding unit analysed confirmed that the level of mating monopolisation in this population is extremely high. Not only did the harem holders achieve the majority of paternities in each harem, but also their success did not decrease with increasing harem size. This confirms previous behavioural results showing that, while the likelihood of monopolisation decreases with harem size in the DEL population, this does not happen on SLI, where the proportion of each holder fertilisation is larger and the number of males achieving at least one fertilisation is smaller (Galimberti *et al.* 2002b). This is mostly due to the large difference in Resource Holding Potential (*RHP* - Parker 1974) between the holders of largest harems and the rest of males. Variation in *RHP* (structural and behavioural) is higher at SLI than at DEL (Fabiani 1996; Galimberti and Fabiani unpublished data). In elephant seals, harem size interacts with individual phenotype to determine reproductive behaviour of males (Modig 1996). In the SLI population, some males are able to get uncontested control of harems with more than one hundred females.

These harem holders are capable of effectively managing the complexity of a social system that comprises hundred of individuals, maintaining the access to females, even in unfavourable socionomic conditions (i.e. very large group of females to control and other males that constantly try to infiltrate the harem). The demographic and social environment is a crucial factor in defining the likelihood of mating monopolisation among males. The higher the *RHP* difference among males, the higher the likelihood for males with larger potential to monopolise the mating season (Dawkins and Krebs 1979). In the SLI population, the social context has been changing since 1999. In these recent years, a reduction in the likelihood of monopolisation has been evident, mainly due to a reduction in *RHP* difference among males. More males similar in *RHP* have been present on the island and hence it has become more difficult for them to maintain the control of a harem for long periods (Galimberti unpublished data).

At SLI, controlling a harem is by far the most rewarding reproductive strategy. Nevertheless, males also pursue other strategies. They associate with harems waiting for opportunities to infiltrate and mate, try to copulate with solitary females, or they follow and mate with departing females. However, these strategies are rarely successful. Females can mate with more than one male each season, but this study showed genetic evidence that the first male seen mating with a female is almost always the father of her pup (77% to 100% of the time in each harem). Since the colony was not under observation 24hrs a day, it is likely that some females recorded at their 'first copulation' had instead already been mated. Similarly, genetic assignments might have been occasionally incorrect (Marshall *et al.* 1998, Coltman *et al.* 1999c). Keeping this in mind, there is strong evidence that securing a female's first copulation is the best strategy to put into effect, and that sperm competition might act towards this direction. In this context, the harem holder strategy clearly allows a male to dominate copulations and to control females during their oestrus period. The other strategies rarely succeed, and males that adopt them seem be forced to a role 'making the best of a bad job' (Krebs and Davies 1993; Andersson 1994; Cunningham and Birkhead 1998).

Copulating with solitary females could in theory be rewarding, but solitary females are extremely rare. In 1996 and 1997, 1.9% and 3.7% of the total females in the colony (n = 527 and 563, respectively) were alone at parturition. Of these, the majority joined a harem before coming into oestrus (80% in 1996 and 67% in 1997), hence only a very small proportion of females remained alone during the whole nursing period (0.38% in 1996 and 1.2% in 1997 of the total number of females). Similarly, at the end of the season, when only few females are left on the beaches, only 1.1-1.8% of them were alone (Galimberti *et al.* 2000a). With so few females available, males pursuing this strategy cannot achieve a high reproductive success.

Mating with departing females does not seem to have relevant success, although further investigation is needed. Almost all females mate before their departure with either the harem holder or other males, and it is likely that those males who mated first would father their offspring (R^2 between *FIR_CO* index and paternity = 0.94; congruence 77-100%). Comparing with DEL, a smaller percentage of female departures are intercepted at SLI than at DEL (41% out of 229 at SLI and 59% out of 120 at DEL). The distance between the harems and the sea is small on SLI, and the departure events tend to be shorter (mean of 15 minutes compared to 31 minutes on DEL) and involve the interaction with secondary males a smaller percentage of times (Galimberti *et al.* 2000a). The situation on SLI seems to be very different from those exhibited in northern elephant seal colonies (see also Discussion in Chapter III), where almost 100% of departing females are intercepted (Le Boeuf and Mesnick 1991a).

Finally, males can associate with a harem and try to infiltrate the female group. This strategy is likely to be more successful in larger harems. In large harems, the holder might not be able to continuously control the situation, and secondary males can take advantage of moments when the vigilance is less strict and achieve some copulations (Le Boeuf 1974). However, at SLI harem holders were extremely efficient, and their success was not correlated with the size of the harem. Although the strategy partly succeeded (9 out of 51 secondary males were recorded mating and 17 were attributed a paternity at 80% confidence level), only three secondary males obtained two paternities. This strategy appears to be the most rewarding among those followed by non-holder males of SLI. Even so, the success achieved is still extremely small if compared with that obtained by holders. However, both DEL and SLI are at the lower end of the variation in harem size and density recorded for southern elephant seals. Harems sizes are larger at South Georgia (mean = 74.2, range 6 to 232, McCann 1980), Macquarie Island (mean = 277, up to 1000, Carrick *et al.* 1962), and Kerguelen (mean = 102, range 5 to 1350, Van Aarde 1980). Therefore, in the case of much larger harems and more packed colonies, the level of monopolisation by the holder may be different and more affected by the number of females breeding in each unit. In northern elephant seal colonies, where individuals breed at higher density and form larger harems with several associated males, the percentages of copulations of the harem holders decrease with the harem size (Le Boeuf 1974; Le Boeuf and Reiter 1988).

Among other polygynous pinnipeds, the level of polygyny does not reach such high values as those reported for southern elephant seals. In southern sea lions (*Otaria byronia*) seasonal *I* estimated from copulations varied from 0.73 (Campagna and Le Boeuf 1988) to 1.31 (Galimberti unpublished data) in two different populations of the Peninsula Valdés, while in Juan Fernández fur seals (*Arctocephalus philippii*) a behavioural estimate of *I* over two seasons equalled 0.73 (Francis and Boness 1991). In grey seals (*Halichoerus grypus*), behavioural data indicated a high variance in male reproductive success (Twiss 1991), but genetic results showed a lower variance in paternity than predicted (Amos *et al.* 1993; Ambs *et al.* 1999; Worthington Wilmer *et al.* 1999). In harbour seals (*Phoca vitulina*), the variance in reproductive success was extremely low, with the population approaching genetic monogamy (seasonal *I* from 0.05 to 0.18). The results partly agreed with the authors' predictions, as aquatic mating is well documented in this species. Besides, Harbour seals are only slightly dimorphic, though males cluster on a scale such that polygyny is possible (Coltman *et al.* 1998). Genetic polygyny far lower than expected was found in the Antarctic fur seal (*Arctocephalus gazella*), where only 35 males out of 243 among two breeding seasons were assigned one paternity, and only four males were assigned two (Gemmell *et al.* 2001).

VI.2.1 Lifetime reproductive success

The variance in male mating success determined in this study can give indications on the variance in male lifetime mating success on SLI. The data represent only an approximation of the actual lifetime reproductive success of the analysed males, due to the short duration of the project compared to the reproductive lifespan of a male. In fact, since males reach reproductive maturity at around 8-10 years of age and live on average 16-18 years, it is possible that those males analysed were successful prior to the start of the project, or that they migrated to successfully breed elsewhere. In this case, the behavioural data available would be an under-estimate of their actual mating success. Preliminary data on lifetime mating success of males of SLI over six seasons (1995-2000) give values of *ENFI* ranging from 0 to 350 (Appendix C). The estimate is calculated on the number of breeding males present each year on land (i.e. it does not consider males on land but not participating in breeding activities, hence it is a conservative estimate). The range is an underestimate since the male (LEO) with the highest total *ENFI* reported (350; seasonal *ENFI*: 107, 118, 125) bred on SLI from 1995 to 1997, but very likely also held a harem before 1995 (the year when the study on island started). Among the other most successful males, two (OVO and TOM) were harem holders for four consecutive years (1995-1998) and achieved total *ENFI*s of 257 and 258 (seasonal *ENFI:* 10, 80, 78, 90 and 32, 36, 80, 110, respectively). A third male (SCAR) bred on SLI for six seasons (1995-2000). The first season he did not hold a harem (and he did not achieve any success), while in subsequent years he always got the control of a harem of 25-108 females. In 1996, his harem was not under behavioural observation hence his total observed *ENFI* is an underestimation of his success. Over the period 1997-2000 his total *ENFI* was 331 (seasonal *ENFI*: 64, 71, 92, 104).

The reproductive history of a successful male seems to generally follow an increasing trend, as his success increases with his experience and he is able to hold larger harems with the passing seasons. Hence LEO, who was highly experienced in 1995 and held a harem of 112 females, was very likely a harem holder in previous seasons. In contrast, OVO was a beta male for the first part of the 1995 season and held a harem of six females only at the end of it, while TOM held a harem of 32 females. Their experience clearly increased from 1995 to 1998, and it likely that 1995 was their first season as holders of harems. Similarly, SCAR was not successful in 1995, when he was a peripheral male of a harem of 99 females. His *ENFI* increased during the consecutive seasons, as he held harems of 25, 40, 83, 65 and 108 females from 1996 to 2000, respectively.

These *ENFI* estimates indicate that males at SLI can have a high seasonal success for many consecutive years; as a result, their lifetime success can be much higher than that reported for the northern elephant seals. In the Californian population, Le Boeuf and Reiter (1988) estimated a lifetime *ENFI* on four cohorts ranging from 0 to 121. The male with the highest estimate (121) achieved an *ENFI* equal or higher than 30 during only two breeding seasons (seasonal *ENFI*: 1, 1, 6, 8, 30, 61, 14). Only two other males had a lifetime success estimate higher than 60 (63 and 97). In both cases they held a harem for only two breeding seasons, during which the estimates of their success were 20 and 26 for the first male and 30 and 66 for the second.

Compared to other mammal species, the elephant seal mating system is probably most similar to that of the red deer (*Cervus elaphus*). In both species breeding is seasonal, males compete vocally and physically for mating, and dominant males monopolise harems of females. In a six-year study, total reproductive success in red deer stags varied from 0 to 32 (Clutton-Brock *et al.* 1988). The maximum value in red deer is hence one tenth the maximum value reported in the SLI population, and clearly indicates that the level of polygyny in the latter is much higher. The level of mating monopolisation in SLI harems is also higher than that reported for lek species (i.e. different measures of inequality in southern elephant seals were always larger when compared with lek species – see Galimberti *et al.* 2002b), and lek species are often considered among the most 'extreme' mating systems, in term of reproductive success variance and potential action of sexual selection (Clutton-Brock 1989). Elephant seals are a classic textbook example of polygyny and sexual selection but such a high level of mating monopolization in the species should be considered far from expected, considering that their harems are at least one order of magnitude larger than harems of other polygynous mammals (Clutton-Brock 1989). The structured social behaviour of elephant seals (McCann 1981), the large size of female groups, the concentrated breeding season (Galimberti and Boitani 1999), and the persistence of non-holders in trying to interact with females, produce a complex social network. Harem holders exhibit an amazing capability to manage such a complex situation.

VI.3 Breeding system and genetic structure

VI.3.1 Female gregariousness and site fidelity

'*Any useful model for the evolution of pinnipeds biology must account for extreme gregariousness, because in the absence of gregariousness during the breeding season, organised polygamy is obviously impossible*' (Bartholomew 1970). The potential for males to mate with many females is highest when receptive females are moderately asynchronous and spatially clumped (Emlen and Oring 1977). A high degree of sociality among females should enable dominant males to control access to oestrus females, and high polygyny and social structuring of females can in turn produce higher levels of kinship within than between groups (Chesser 1991b). This study demonstrated that the high degree of polygyny in southern elephant seals does not lead to any genetic sub-structure within the population. Furthermore, site fidelity and philopatry were not predictors of the relatedness patterns in the population.

Female gregariousness during the breeding season is extremely high in the genus *Mirounga* and in many otariids, but theoretical predictions on kin selection have never found clear evidence in pinnipeds. For example, some species exhibit fostering behaviour, but high levels of relatedness were not found between pups and fostering females in harbour seals (Schaeff *et al.* 1995). What seems most probable is that females that become separated from their pups (or loose them at birth) might attempt to foster pups that are alone or also with other females. On SLI, females that lose their pup or that do not give birth remain in the harem throughout the breeding season and often try to approach other females' pups. In one case, a female that lost her pup at birth fostered a weaned pup after his mother left the harem. Although very rarely, females can give birth to two pups but do not always nurse both of them. In these cases, the pup deserted by the mother can either be expelled from the harem or fostered by another female. These occasional behaviours do not seem to be the ultimate factors that promote female gregariousness and site fidelity.

Harem grouping can act as a defence from aggressive male mating behaviour (Cassini 1999). Harassment by males may have important consequences in the evolution of phenotypic and behavioural traits by promoting an arms race between the sexes (Clutton-Brock *et al.* 1992; Clutton-Brock and Parker 1995). Through the dilution effect of grouping and the exclusion of marginal males by higher-ranking males, females in a group can suffer less harassment. In southern elephant seals, females breeding in a harem experience lower level of harassment than solitary females, and females in larger harems are less frequently harassed than females breeding in smaller groups (Galimberti *et al.* 2000a). Galimberti *et al.* (2000a) analysed 65 harems from DEL and SLI, ranging in size from 3 to 168 females, therefore their results provide a strong indication of the positive effect of female clustering on harassment reduction (i.e. in a multiple regression, weighed harassment rate was not related to the number of associated males but it was negatively related to harem size: *beta* = -0.6, $P = 0.0072$). Although sometimes intense, female harassment on SLI does not affect the likelihood of successfully weaning a pup (Galimberti *et al.* 2000a). However, in other populations and species, where harems are larger and beaches more crowded, it can involve serious injures to females and reduce their reproductive success (Le Boeuf and Mesnick 1990a; Boness *et al.* 1995). Because it can involve severe costs for female fitness, harassment can therefore exert a significant selective pressure in shaping the female behaviour and hence the pinniped mating systems (Cassini 1999, 2000).

Site fidelity and philopatry are correlated with female gregariousness. From a genetic point of view, females returning to the same site to breed and aggregating in groups can show a higher level of kinship among each other than between individuals of other groups (Chesser 1991a). Site fidelity is an important element of mammal life history strategies (Greenwood 1980) and is typical of the breeding biology of many land-breeding pinnipeds (e.g. *Callorhinus ursinus*: Baker *et al.* 1995; *Mirounga leonina*: Nicholls 1970, Lewis *et al.* 1996; *Mirounga angustirostris*: Reiter *et al.* 1981; *Halichoerus grypus*: Pomeroy *et al.* 1994; *Phoca vitulina*: Stanley *et al.* 1996, Schaeff *et al.* 1999).

Apart from a generalized tendency to return to the same breeding colony, female southern elephant seals from Sea Lion Island consistently choose the same part of the colony for breeding during consecutive seasons. Of 646 females, 84% gave birth within 500 m of their parturition location in the previous year (70% returned to the same breeding zone and 37% to the same area). The degree of site fidelity of Sea Lion Island's females is higher than that recorded for the elephant seals of the close colony of DEL (the 3 km criterion adopted Lewis *et al.* (1996) will include almost 100% of the females if applied to the Sea Lion Island's population). Similarly, it is comparable with the 71% reported for the northern species (Reiter *et al.* 1981).

The mating system of a species can strongly influence female movements and patterns of individual site fidelity. In the territorial northern fur seal, females return to the same breeding site with an almost metric precision, favoured by the territorial social organization and the topography of breeding sites (Gentry 1998). Such a precise fidelity is not achievable in species that do not have a territorial social system. The level of fidelity exhibited by southern elephant seals of SLI is surprisingly high if the structure of their mating system is considered. Females on SLI come ashore on the beach and join a harem where they will stay for the rest of the breeding season (only 20% out of 1151 females changed harem between arrival and parturition). Therefore, their nursing location depends on the location (and presence) of the harem, which, in turn, is constrained by social factors (i.e. number and dispersion of the other females in the harem and the capability of the harem holder to herd females). Moreover, SLI breeding habitat is extremely uniform. It consists of open long and large beaches with no particular topographical features that seem to attract females to specific areas of the colony, as has been reported for the grey seal (Twiss *et al.* 2000; Pomeroy *et al.* 2001), or to preclude the formation of a harem. Large parts of the beaches are free of females and the distance between adjacent harems varied from 130 to 415 meters in 1997 (distances between harem centres, Galimberti unpublished data). It is thus remarkable that, in this social and spatial environment, females are able to give birth in the same area in different years.

At the colony of SLI, the higher level of mean relatedness among females than among males supports the behavioural evidence of larger returning rates for females than for males. However, the tendency of females to return to the same site of the previous year does not lead to a higher level of kinship within harems. In fact, among those females returning to the same harem, only seven pairs out of 220 showed a kinship level larger than 0.4, and among the other females no relationship was detectable. These results illustrate that the mating system acts as a dilution factor on the distribution of relatedness. Although females tend to come back to the same part of the colony to reproduce, it appears that the size of the harems is sufficient to mitigate the effect of philopatry and fidelity.

The hypothesis of genetic mate selection, which has been advanced for grey seals (Amos *et al.* 2001), does not seem to be supported by the results of this study. Females were in fact not found either more or less genetically similar to their holder than to other males. However, the genetic data covered only three years and it was not possible to compare partners of the same females among seasons, as Amos *et al.* (2001) did with 48 grey seal females. Nevertheless, the behavioural and genetic results suggest that females do not choose their harem in response to the genotypic relatedness they have with the harem holder (i.e. the male with whom they are likely to mate). Once arrived on land, females may move harem before parturition and, if they do so, they tend to move towards larger harems (70% out of 207 females that move harem – see Discussion in Chapter IV). Moreover, a harem can change its holder during the season, females almost never move after giving birth (unless they are isolated females and join a harem after parturition), and there is no indication that they follow their holder to other harems (Galimberti et al. 2000a). Females tend to choose the same part of the colony of previous years and their precision tends to increase with their experience (Fabiani *et al.* in prep.). However, their grouping does not seem to be the result of 'genetic decisions' (i.e. towards higher kinship among females or towards particular related males) but it seems instead a strategy mainly adopted to avoid harassment from inexperienced males.

VI.3.2 Dispersal patterns

The degree of faithfulness shown by mammals and birds to their natal or breeding site is often gender-dependent (Greenwood 1980). One likely consequence of such asymmetric dispersal is that a species may exhibit different patterns of geographic population structure at genes with biparental transmission (i.e. nuclear markers such as microsatellites) versus those at which transmission occurs through only one sex (i.e. mtDNA and Y chromosome for mammals) (Avise 1994). In most mammalian species, dispersal is male-biased, and higher female fidelity and more frequent male dispersal are also frequently suggested from data for cetaceans (e.g. for *Megaptera novaeangliae*: Baker *et al.* 1990; *Delphinapterus leucas*: O'Corry-Crowe *et al.* 1997;

Physeter macrocephalus: Lyrholm *et al.* 1999; *Phocoenoides dalli*: Escorza-Teviño and Dizon 2000).

The genetic analysis of elephant seals of SLI and EI supported the concept of female fidelity to breeding site, and showed genetic differentiation among colonies both within and between stocks. However, female mediated gene flow was present among colonies, with the apparent exception of Peninsula Valdés, confirming the results from Hoelzel *et al.* (1993). In particular, differentiation at nuclear markers was higher than at mitochondrial markers, suggesting a difference in the pattern of breeding dispersal between sexes.

Differences in the characteristics of the mtDNA and nuclear markers, such as mutation rate and/or rate of genetic drift, might also be responsible for the difference in geographical structure found between them. The greater sensitivity of mtDNA to genetic drift is usually explained by two factors: its smaller effective population size and its higher rate of mutation. Given its haploid nature and its maternal inheritance, mtDNA is 1/4 times the number of heritable copies and so potentially 1/4 the diversity of any nuclear marker. Therefore, the effective size for mtDNA markers is four times lower than that for nuclear markers. A smaller effective size increases the rate of genetic drift and therefore the rate at which populations are expected to exhibit differences in allele frequencies. In southern elephant seals female effective population size has been calculated as being roughly equal to the population effective size, hence the rate of drift of mtDNA is expected to be twice that of the nuclear genome (Slade 1997). The higher mutation rate of the mtDNA can also generate greater subdivisions for two major reasons. First, the population would show a larger number of alleles at lower frequency than those exhibited by nuclear genes. Second, isolated populations would have more chances to contain private alleles (i.e. unique alleles that can occur within each population after separation). However, these differences between mitochondrial and nuclear genomes do not appear to be sufficient to explain the differences in the genetic structure found in this study. Thus, a difference in the rate of gene flow for males and females seems to be the major factor in determining the different patterns of genetic differentiation.

Previous studies, revealing three main stocks among southern elephant seals (Slade *et al.* 1998; Hoelzel *et al.* 1993), were confirmed in this research, and the populations of EI and SLI generally fit into the South Georgia stock structure. From the analysis of mitochondrial markers, striking differentiation was found between EI and SLI and the close PV, while a few haplotypes were shared with SG. Given the presence of only one mtDNA lineage among the Argentinean seals, Hoelzel *et al.* (2001) suggested a founding event on the peninsula with no further significant female recruitment and a more frequent dispersal by males between the peninsula and the close islands. In fact, although differentiation between PV and other colonies was also present at nuclear markers, patterns of variation were similar within colonies. It is however surprising that the level of differentiation between PV and SLI was very similar to that of PV from SG (ϕ_{ST} = 0.53 and 0.58, respectively). The shorter geographical distance between PV and SLI does not seem to have lead to more frequent movements of individuals between the two colonies than between SG and PV.

Although no males or females marked on PV have been resighted as breeding on SLI since 1995 during an extremely well monitored study (Galimberti and Boitani 1999), the PV colony is much bigger and individuals more dispersed than on SLI (Campagna and Lewis 1992). The topography of PV does not allow a constant monitoring of the entire breeding population, i.e. not all individuals breeding on the peninsula are tagged or otherwise marked, and daily censuses are carried out only on a small part of the colony. It is then possible that unmarked seals on SLI have not been identified as coming from PV, or that marked seals from SLI have not been resighted on PV. Nevertheless, a female was recorded breeding both on SLI and PV among six breeding seasons. She was first tagged as an adult breeding female on SLI in 1995, she skipped the breeding season on the island in 1996 (and she was not resighted elsewhere that year) and was recorded breeding again in the colony in 1997. After 1997 she did not breed anymore on SLI, but in 2000 she was resighted breeding on PV, at the colony of Punta Delgada (DEL).

Despite the extensive mitochondrial variation (ϕ_{ST} = 0.5), genetic evidence of male mediated gene flow was also found between SLI and MQ, as an adult male (BLOB) showed the same haplotype as a seal belonging to the very well defined MQ lineage (Fabiani *et al.* in press). Elephant seals have been found to move considerable distances during their foraging-moulting trips. Individuals from South

Georgia travelled up to 3000 km from their breeding to foraging sites (McConnell and Fedak 1996), while a juvenile tagged on MQ has been resighted moulting on Peter 1 ØY, some 5200 km away from Macquarie (Hindell and McMahon 2000). Among pinnipeds, northern elephant seals can cover up to 11000 km during their round-trip post-breeding migrations (Stewart and De Long 1994), while harp seals can travel 4000 km from feeding to breeding grounds (Stevick *et al.* 2002). Individual variation in migration patterns is evident; however, up to now, a breeding migration distance of as long as 8000 km has never been recorded for any seal. This distance appears to be closer to those covered by whales during their migrations from feeding to breeding areas (6500 in sperm whales, 8000 km in humpback whales, 9000 km in grey whales, see Stevick *et al.* 2000 for a review).

It is very likely that BLOB was born on MQ. A stepping-stone migration (i.e. migration only between neighbour populations) is possible but less likely. The MQ lineage is very well supported and defined by two fixed differences. It is unique to MQ, with the only exception of BLOB out of 154 sampled individuals coming from five populations. Given the pattern of genetic diversity shown by the six colonies analysed, and the patterns of fidelity and dispersal in elephant seals (i.e. males disperse more than females and females exhibit higher social and geographic fidelity), the most parsimonious interpretation is that the male travelled from MQ to SLI. If his mother had migrated to different populations, she would have passed her haplotype onto her offspring and more copies would have been available for sampling. Moreover, a female would leave copies of her haplotypes in different populations as she migrates and those offspring could also disperse to other populations. Hence, females have a greater potential to homogenise populations through dispersal, since their migration impacts not only females, but also the co-migrating offspring regardless of its sex (Tiedemann *et al.* 2000). If, despite this, females exhibit higher levels of genetic structure, it would only be 'conservative' to assume that dispersal is male biased, meaning that males are moving, mating and homogenising the populations (Escorza-Treviño and Dizon 2000).

If female dispersal can have a stronger impact on the genetic structure of a population than male dispersal (Tiedemann *et al.* 2000), their effects are strongly influenced by the population mating system. In case of high polygyny, not only will just a few males reproduce, but also they will pass their genes to a high number of offspring, effectively increasing the genetic uniformity in the population. The effects of the mating system will, in turn, co-interact with life history traits (i.e. growth, mortality and productivity patterns), with 'condition' and 'quality' of reproductive individuals (McNamara and Houston 1996), and finally contribute to the genetic structure of the species.

The male BLOB was on SLI for only two seasons (1995 and 1996). He successfully reproduced only in 1996, gaining the control of a harem and fathering at least 18 offspring. His exceptional migration may indicate that even populations separated by wide genetic and geographic distances can be linked. His migration combined with his reproductive performance is an example of the potential homogenising effect that dispersal and mating system can have on the genetic patterns of a population. At the same time, his dispersal might have contributed to his reproductive success. In fact, he might have found better demographic and social conditions on SLI than on MQ, as on Macquarie Island harems are on average some five times larger than on SLI (270 vs 45 females), and density of individuals much more elevated (McCann 1980).

Concluding, the comparison of behavioural and genetic data in assessing genetic and dispersal patterns of a population, together with information from mating system studies, provide important evidences. First, direct behavioural studies on current populations may provide a misleading picture of the geographic distribution of genetic patterns, because they fail to access the evolutionary aspects of population dispersion and structural organisation revealed by genes. Second but no less important, geographic distribution of genetic markers may provide a misleading picture of current dispersal, gene flow or mating patterns, because they retain records of evolutionary events and demographic contexts that may differ from those of the present (Avise 1994). Therefore, a full understanding of the population genetic structure of a species, and of those factors significant for its evolutionary mating patterns, requires investigations from both behavioural and genetic perspective.

VII. References

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APPENDIX A – Paternity Program CERVUS

A general introduction (from Marshall *et al.* 1998) to the likelihood analysis implemented in the program CERVUS is presented here. More detailed description on the program can be found in Marshall *et al.* (1998) and in the references therein.

If genetic data were perfect, a mismatch between a male and a putative offspring should be treated as an evidence for exclusion of that male from paternity. However, a mismatch could result either from a genuine non-relationship or from a laboratory typing error. When microsatellites are used, mutations and null alleles (Pemberton *et al.* 1995) may also generate mismatches between genuine relatives at measurable frequencies. Because a series of loci are used in parentage analysis, the probability of at least one mismatch between true relatives due to typing error across all loci and individuals involved in a parentage test can be relatively high, even when the frequency of typing error at any one locus is low.

Probability of exclusion

Parentage testing can take place either in the presence or in the absence of genetic information from the other parent. CERVUS calculates the average probability of excluding a single randomly selected unrelated candidate from parentage for each of these two cases. It also calculates the combined exclusion probability across all loci: this represents the combined power of the set of loci of excluding a single randomly-chosen unrelated individual from parentage at one or more loci.

The exclusion probability (P_l) for each locus and for the set of loci was calculated using the allele frequency option of CERVUS. Two exclusion probabilities were calculated. One is the probability to exclude a randomly-selected male from paternity given only the genotype of the offspring. The second is the probability of excluding a randomly-selected male from paternity when both the offspring and the mother are sampled.

As in Marshall *et al.* (1998):
for one locus l with K codominant alleles and given only the genotype of the offspring:

$$P_l = a_1 - 4a_2 + 4a_3 - 3a_4 + 2a_2^2$$

for one locus l with K codominant alleles and given the genotypes of the offspring and other mother :

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

where

$$a_n = \sum_{i=1}^{k} p_i^n$$

The combination exclusion probability across n independently inherited loci, P, was calculated as performed in the program:

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

Error rate

The likelihood approach of CERVUS allows taking account of potential imperfections of the data, and defines an error as a replacement of the true genotype at a particular locus in n individual with a random genotype (Marshall *et al.* 1998). Following this approach, a true parent that mismatches at one or two loci with the offspring can still often be identified as the most-likely parent, providing that the power of loci is reasonably high (i.e. large number of loci and high level of polymorphism).

Allowing for typing errors also dramatically reduces the impact of two other possible causes of mismatches in parent-offspring relationships: mutations and null alleles. While it is not statistically ideal to treat mismatches arising from mutations and null alleles as if they were mismatches arising from typing errors, this is a very much better approximation than using such mismatches as a basis for parentage exclusion (Marshall *et al.* 1998). Unlike other simulation parameters (see after), the error rate is not only used to generate simulated genetic data, but also used directly in the likelihood calculations, both in the simulation and in parentage analysis.

Calculation of paternity likelihood

Assuming mating is random and that the mother's genotype is known, CERVUS calculates the likelihood ratio for each male on each pup as the ratio of two alternative hypotheses: (1) the mother and the alleged father are parents of the pup ($L(H_1)$); (2) the mother is the parent and the father is not the true father but a randomly chosen individual from the population ($L(H_0)$):

$$L(H_1)/L(H_0) = \frac{P(g_p/g_m,g_a).P(g_m).P(g_a)}{P(g_p/g_m).P(g_m).P(g_a)}$$

where g_p, g_m and g_a represent the pup, maternal, and alleged paternal genotypes. This simplifies to:

$$L(H_1)/L(H_0) = \frac{P(g_p/g_m,g_a)}{P(g_p/g_m)}$$

and thus represents how much more likely it is that the alleged father, rather than an arbitrary male, passed his genes to the offspring (Marshall *et al.* 1998).

Because several unlinked marker loci are used in the analysis, the program calculates likelihood ratios for each male at each locus and takes the natural logarithm of the product across all loci. This is called the LOD score. LOD score of zero implies that the assumed father is as likely to be the father of the offspring as a randomly selected male; a positive LOD score implies that he is more likely to be the father than a randomly selected male; a negative LOD score may occur if the assumed father and offspring share a particularly common set of alleles (Marshall *et al.* 1998). Inference about paternity is made by CERVUS according to the magnitude of the difference between the LOD of the most-likely and the next most-likely father. It generates a statistic, , defined as the difference in LOD between the top two candidate males, and uses a simulation based on the observed allele frequencies to determine the statistical significance of the value generated for each paternity (see chapter II for details on the simulation parameters used).

APPENDIX B – Paternity assignment within harems

The results of the paternity assignment for each harem are reported. In each table, the first most-likely father (or the two most-likely fathers, i.e. single male or two males with the highest LOD values) is indicated for each pup. The male with the highest LOD and showing a confidence level ≥ 80% for the paternity was recognised as the 'genetic father' and used in the analyses. In case CERVUS identified a father at confidence level <80%, the paternity was considered not assigned and was not used in the analyses. Mother-pup pairs for which no male could be identified by the program as father were also not used in the analyses.

Pup: ID of the pup

Pup loci: number of loci screened for the pup

Known mother: ID of the known mother of the pup

P-KM mism: number of mismatches between the pup and the mother

Prob non-exclusion: 1- probability of exclusion (i.e. probability of excluding a randomly-selected male from paternity when both the offspring and the mother are sampled)

Candidate father: ID of the male identified as the most likely father by CERVUS (or of the two males identified as the two most likely fathers). The male identified as the first most likely father by the program was considered in the analyses as the 'genetic father', and it is shown in bold type. In some paternities, the 'behavioural father' is assigned as the second most likely father, without mismatches and with a LOD very similar to the LOD of the first most likely father (see Discussion in Chapter III). In these cases, the 'behavioural father' is indicated in bold italic type.

CF status: seasonal status of the male:
s holder: seasonal holder of the harem (he held the control of the harem for the longest period)
t holder: temporary holder of the harem (he held the control for a short period)
oh holder: other harem holder (he was the holder of a different harem)
secondary: secondary male (either peripheral or marginal male)
tertiary: tertiary male (he was never observed associated to a harem)

Con lev: confidence level for the paternity:
*: 95%
+: 80%
-: ≤80% (but still identified as the most likely father by the program)
•: no father identified

LOD: LOD score (see Appendix A)

Delta: difference in LOD between the top two candidate males (see Appendix A)

CF loci: number of loci screened for the candidate father

P-CF mism: number of mismatches between the pup and the candidate father. In only two cases (pups V3602I and V3888I) the paternity was assigned with one mismatch. In both cases, the program explained the mismatch with the presence of a null allele in the candidate father.

Harem RUB96

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3677I	7	V0092D	7	0	3.88E-02	**PROBO**	s holder	+	2.64E+00	6.44E-01	7	0
V3677I	7	V0092D	7	0	3.88E-02	RON			1.99E+00	0.00E+00	7	0
V3384I	7	R419I	7	0	3.93E-02	**PROBO**	s holder	*	2.58E+00	2.16E+00	7	0
V3384I	7	R419I	7	0	3.93E-02	TESTO			4.21E-01	0.00E+00	7	1
V3563I	7	R432D	7	0	7.63E-02	**TONNO**	secondary	+	2.55E+00	6.86E-01	7	0
V3563I	7	R432D	7	0	7.63E-02	***PROBO***	*s holder*		1.87E+00	0.00E+00	7	0
V3755D	7	R628D	7	0	3.93E-02	***PROBO***	*s holder*	–	2.55E+00	1.59E-02	7	0
V3755D	7	R628D	7	0	3.93E-02	RON			2.53E+00	0.00E+00	7	0
V3618D	7	A201I	7	0	1.36E-01	**TONNO**	secondary	+	2.14E+00	6.86E-01	7	0
V3618D	7	A201I	7	0	1.36E-01	***PROBO***	*s holder*		1.45E+00	0.00E+00	7	0
G409I	7	V0201D	7	0	1.92E-03	FAT	t holder	*	6.65E+00	4.79E+00	7	0
G409I	7	V0201D	7	0	1.92E-03	ICS			1.86E+00	0.00E+00	7	1
V3682I	7	V0265I	7	0	2.95E-02	**RON**	terziary	+	2.78E+00	8.34E-02	7	0
V3682I	7	V0265I	7	0	2.95E-02	***PROBO***	*s holder*		2.70E+00	0.00E+00	7	0
G206D	7	V0325I	7	0	1.62E-03	**PROBO**	s holder	*	5.90E+00	5.90E+00	7	0
G206D	7	V0325I	7	0	1.62E-03	MINO			-6.55E-01	0.00E+00	7	2
V3741I	7	V0628I	7	0	3.49E-03	**PROBO**	s holder	*	5.14E+00	5.14E+00	7	0
V3741I	7	V0628I	7	0	3.49E-03	GEO			-1.74E+00	0.00E+00	7	2
G118I	7	V0836I	7	0	1.21E-03	**FAT**	t holder	*	7.41E+00	7.41E+00	7	0
G159I	7	V0920I	7	0	3.68E-04	**PROBO**	s holder	*	7.86E+00	7.86E+00	7	0
G159I	7	V0920I	7	0	3.68E-04	PONGO			-2.31E+00	0.00E+00	7	3

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G323I	7	V0955D	7	0	2.62E-02	**PROBO**	s holder	*	4.07E+00	2.29E+00	7	0
G323I	7	V0955D	7	0	2.62E-02	GEO			1.78E+00	0.00E+00	7	1
V3816I	7	V1057D	7	0	2.03E-02	**PROBO**	s holder	*	3.42E+00	2.26E+00	7	0
V3816I	7	V1057D	7	0	2.03E-02	CIOP			1.16E+00	0.00E+00	7	0

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Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G105I	7	A177D	7	0	1.02E-03	**BLOB**	s holder	*	6.37E+00	6.37E+00	7	0
G105I	7	A177D	7	0	1.02E-03	IPIR			-1.46E+00	0.00E+00	7	1
G229I	7	A178I	7	0	2.78E-05	**BLOB**	s holder	*	9.58E+00	9.58E+00	7	0
G229I	7	A178I	7	0	2.78E-05	LEO			-1.41E+00	0.00E+00	7	1
G331I	7	A327D	7	0	8.40E-03	**IGOR**	secondary	*	4.60E+00	4.60E+00	7	0
G331I	7	A327D	7	0	8.40E-03	TOM			-3.39E+00	0.00E+00	7	1
G243I	7	A355D	7	0	1.32E-04	**BLOB**	s holder	*	8.07E+00	8.07E+00	7	0
G243I	7	A355D	7	0	1.32E-04	MO			-3.70E+00	0.00E+00	7	1
G461I	7	A511I	7	0	3.77E-05	**BLOB**	s holder	*	9.18E+00	7.41E+00	7	0
G461I	7	A511I	7	0	3.77E-05	SOB			1.77E+00	0.00E+00	7	1
V3502D	7	R434D	7	0	8.00E-05	**BLOB**	s holder	*	8.68E+00	8.68E+00	7	0
V3502D	7	R434D	7	0	8.00E-05	ALVI			-9.49E-01	0.00E+00	7	0
V3714I	7	R499D	7	0	1.47E-04	**BLOB**	s holder	*	8.26E+00	8.26E+00	7	0
V3714I	7	R499D	7	0	1.47E-04	LEO			-2.91E+00	0.00E+00	7	2
V3734I	7	V0127I	7	0	3.94E-03	**BLOB**	s holder	*	5.36E+00	4.66E+00	7	0
V3734I	7	V0127I	7	0	3.94E-03	LAMPO			7.00E-01	0.00E+00	7	1
V3727I	7	V0139D	7	0	5.17E-04	**BLOB**	s holder	*	6.66E+00	6.66E+00	7	0
V3727I	7	V0139D	7	0	5.17E-04	LEO			-8.53E-01	0.00E+00	7	2
V3602I	7	V0146D	7	0	1.76E-04	**SOB**	secondary	+	4.06E+00	3.20E-01	7	1
V3602I	7	V0146D	7	0	1.76E-04	***BLOB***	*s holder*		3.74E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3781I	7	V0156I	7	0	6.33E-04	**BLOB**	s holder	*	6.67E+00	6.67E+00	7	0
V3781I	7	V0156I	7	0	6.33E-04	SOB			-8.16E-01	0.00E+00	7	2
V3778I	7	V0212D	7	0	3.68E-04	**BLOB**	s holder	*	7.21E+00	7.21E+00	7	0
V3778I	7	V0212D	7	0	3.68E-04	ALVI			-2.61E+00	0.00E+00	7	0
V3575D	7	V0267D	7	0	4.93E-04	**BLOB**	s holder	*	7.91E+00	7.91E+00	7	0
V3575D	7	V0267D	7	0	4.93E-04	MO			-4.86E+00	0.00E+00	7	1
V3839I	7	V0277I	7	0	3.46E-05	**BLOB**	s holder	*	9.40E+00	9.40E+00	7	0
V3839I	7	V0277I	7	0	3.46E-05	ALVI			-2.95E-01	0.00E+00	7	1
V3966I	7	V0498I	7	0	2.43E-04	**BLOB**	s holder	*	2.99E+00	2.53E+00	7	0
V3966I	7	V0498I	7	0	2.43E-04	INGO			4.65E-01	0.00E+00	7	2
V3774I	7	V0549D	7	0	5.70E-03	**BLOB**	s holder	*	4.88E+00	4.88E+00	7	0
V3774I	7	V0549D	7	0	5.70E-03	LEO			-1.13E+00	0.00E+00	7	2
G194I	7	V0686D	7	0	1.64E-03	**BLOB**	s holder	+	5.75E+00	2.03E-01	7	0
G194I	7	V0686D	7	0	1.64E-03	SOB			5.55E+00	0.00E+00	7	0
G210I	7	V0709D	7	0	6.43E-05	**BLOB**	s holder	*	9.05E+00	9.05E+00	7	0
G210I	7	V0709D	7	0	6.43E-05	LAMPO			-1.30E+00	0.00E+00	7	2
G462I	7	V0724D	7	0	9.63E-05	**BLOB**	s holder	*	8.27E+00	8.27E+00	7	0
G462I	7	V0724D	7	0	9.63E-05	DON			-5.12E-01	0.00E+00	7	1
V3893I	7	V0872D	7	0	1.69E-04	**BLOB**	s holder	*	8.11E+00	8.11E+00	7	0
V3893I	7	V0872D	7	0	1.69E-04	LEO			-3.79E+00	0.00E+00	7	1

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Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G126I	7	A047D	7	0	3.48E-02	IGOR	secondary	–	3.64E+00	4.40E-02	7	0
G126I	7	A047D	7	0	3.48E-02	***OZ***	*s holder*		3.59E+00	0.00E+00	7	0
G458I	7	A345I	7	0	4.46E-02	**TOM**	t holder	+	3.03E+00	6.32E-01	7	0
G458I	7	A345I	7	0	4.46E-02	7			2.40E+00	0.00E+00	7	0
V3413D	7	R470D	7	0	4.67E-04	**OZ**	s holder	*	6.59E+00	4.83E+00	7	0
V3413D	7	R470D	7	0	4.67E-04	IGOR			1.76E+00	0.00E+00	7	0
V3566D	7	R498I	7	0	8.61E-04	**TOM**	t holder	*	7.20E+00	7.20E+00	7	0
V3566D	7	R498I	7	0	8.61E-04	IGOR			-7.47E+00	0.00E+00	7	2
G196I	7	R518I	7	0	9.81E-03	**OZ**	s holder	+	4.95E+00	6.55E-01	7	0
G196I	7	R518I	7	0	9.81E-03	TEO			4.29E+00	0.00E+00	7	0
V3883I	7	R551I	7	0	3.53E-02	**OZ**	s holder	+	3.26E+00	2.82E-01	7	0
V3883I	7	R551I	7	0	3.53E-02	PAZZO			2.98E+00	0.00E+00	7	0
V3884I	7	R570I	7	0	1.68E-02	**OZ**	s holder	+	4.57E+00	3.89E-01	7	0
V3884I	7	R570I	7	0	1.68E-02	TEO			4.18E+00	0.00E+00	7	0
V3793D	7	R572D	7	1	7.24E-03	**OZ**	s holder	+	1.21E+00	3.64E-01	7	0
V3793D	7	R572D	7	1	7.24E-03	BAU			8.46E-01	0.00E+00	7	3
G110I	7	R637I	7	0	1.62E-02	**OZ**	s holder	+	4.35E+00	7.27E-01	7	0
G110I	7	R637I	7	0	1.62E-02	TEO			3.63E+00	0.00E+00	7	0
V3690I	7	R672D	7	0	6.16E-02	IGOR	secondary	–	2.68E+00	4.12E-02	7	0
V3690I	7	R672D	7	0	6.16E-02	***OZ***	*s holder*		2.64E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3385I	7	V0090D	7	0	7.68E-04	**OZ**	s holder	*	5.85E+00	4.76E+00	7	0
V3385I	7	V0090D	7	0	7.68E-04	ICS			1.08E+00	0.00E+00	7	0
V3852D	7	V0103I	7	0	1.16E-02	**7**	secondary	*	3.84E+00	2.25E+00	7	0
V3852D	7	V0103I	7	0	1.16E-02	TONNO			1.59E+00	0.00E+00	7	1
G205I	7	V0126I	7	0	4.10E-02	**OZ**	s holder	+	3.39E+00	2.88E-01	7	0
G205I	7	V0126I	7	0	4.10E-02	69			3.10E+00	0.00E+00	7	0
G122I	7	V0129I	7	0	5.48E-04	**OZ**	s holder	*	6.98E+00	5.51E+00	7	0
G122I	7	V0129I	7	0	5.48E-04	TEO			1.48E+00	0.00E+00	7	0
V3567D	7	V0130I	7	0	7.67E-04	**OZ**	s holder	*	6.70E+00	3.54E+00	7	0
V3567D	7	V0130I	7	0	7.67E-04	INGO			3.16E+00	0.00E+00	7	0
V3721I	7	V0137I	7	0	2.03E-04	**OZ**	s holder	*	7.64E+00	4.46E+00	7	0
V3721I	7	V0137I	7	0	2.03E-04	TEO			3.18E+00	0.00E+00	7	0
V3985I	7	V0141I	7	0	5.07E-02	**2C**	secondary	+	2.32E+00	6.55E-01	7	0
V3985I	7	V0141I	7	0	5.07E-02	7			1.67E+00	0.00E+00	7	0
G074I	7	V0209I	7	0	2.24E-02	**INGO**	terziary	+	4.77E+00	6.29E-01	7	0
G074I	7	V0209I	7	0	2.24E-02	***OZ***	*s holder*		4.14E+00	0.00E+00	7	0
V3764I	7	V0275I	7	0	1.24E-04	**OZ**	s holder	*	8.23E+00	4.90E+00	7	0
V3764I	7	V0275I	7	0	1.24E-04	TEO			3.33E+00	0.00E+00	7	0
V3516D	7	V0330D	7	0	1.03E-01	**CIOP**	terziary	+	2.85E+00	6.80E-01	7	0
V3516D	7	V0330D	7	0	1.03E-01	TONNO			2.17E+00	0.00E+00	7	0
G098I	7	V0410I	7	0	1.05E-01	**TONNO**	secondary	–	2.25E+00	4.10E-02	7	0
G098I	7	V0410I	7	0	1.05E-01	BLIX			2.21E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G493I	7	V0437D	7	0	2.95E-03	**TOM**	t holder	*	5.86E+00	5.86E+00	7	0
G493I	7	V0437D	7	0	2.95E-03	BOLLO			-8.98E+00	0.00E+00	7	4
V3885D	7	V0461I	7	0	1.00E-03	**OZ**	s holder	*	6.19E+00	4.80E+00	7	0
V3885D	7	V0461I	7	0	1.00E-03	SONNI			1.39E+00	0.00E+00	7	0
G155I	7	V0472I	7	0	2.22E-03	**FAT**	oh holder	*	6.65E+00	6.65E+00	7	0
V3905D	7	V0499D	7	0	2.08E-02	**OZ**	s holder	+	4.57E+00	9.66E-01	7	0
V3905D	7	V0499D	7	0	2.08E-02	PAZZO			3.60E+00	0.00E+00	7	0
G255I	7	V0507I	7	0	9.27E-04	**OZ**	s holder	*	6.51E+00	4.72E+00	7	0
G255I	7	V0507I	7	0	9.27E-04	INGO			1.79E+00	0.00E+00	7	0
G492I	7	V0515D	7	0	2.74E-03	**TOM**	t holder	*	6.53E+00	5.85E+00	7	0
G492I	7	V0515D	7	0	2.74E-03	7			6.85E-01	0.00E+00	7	0
V3974I	7	V0519I	7	0	5.43E-02	**OZ**	s holder	+	3.63E+00	1.26E+00	7	0
V3974I	7	V0519I	7	0	5.43E-02	GRIG			2.37E+00	0.00E+00	7	0
G294I	7	V0522D	7	0	4.84E-02	**TOM**	t holder	+	3.10E+00	6.14E-01	7	0
G294I	7	V0522D	7	0	4.84E-02	BLIX			2.48E+00	0.00E+00	7	0
GA08I	7	V0648I	7	0	3.53E-03	**TOM**	t holder	*	5.70E+00	5.19E+00	7	0
GA08I	7	V0648I	7	0	3.53E-03	BLIX			5.06E-01	0.00E+00	7	0
G163I	7	V0701D	7	0	8.61E-04	**TOM**	t holder	*	7.25E+00	7.25E+00	7	0
G163I	7	V0701D	7	0	8.61E-04	IGOR			-8.09E+00	0.00E+00	7	2
V3517I	7	V0710D	7	0	1.26E-02	**OZ**	s holder	+	4.64E+00	6.93E-01	7	0
V3517I	7	V0710D	7	0	1.26E-02	TEO			3.94E+00	0.00E+00	7	0
G164I	7	V0789I	7	0	4.65E-03	**TOM**	t holder	*	5.75E+00	5.75E+00	7	0
G164I	7	V0789I	7	0	4.65E-03	IGOR			-5.91E+00	0.00E+00	7	1

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3877I	7	V0832I	7	0	5.66E-04	**OZ**	s holder	*	6.72E+00	4.86E+00	7	0
V3877I	7	V0832I	7	0	5.66E-04	FILM			1.86E+00	0.00E+00	7	1
V3802I	7	V0929I	7	0	3.72E-04	**OZ**	s holder	*	7.54E+00	4.23E+00	7	0
V3802I	7	V0929I	7	0	3.72E-04	INGO			3.31E+00	0.00E+00	7	1
G192I	7	V0985D	7	0	4.96E-03	**SOB**	secondary	*	4.26E+00	2.35E+00	7	0
G192I	7	V0985D	7	0	4.96E-03	ILOB			1.91E+00	0.00E+00	7	1
G127I	7	V1082D	7	0	1.51E-02	**OZ**	s holder	+	4.33E+00	6.40E-01	7	0
G127I	7	V1082D	7	0	1.51E-02	TEO			3.69E+00	0.00E+00	7	0
GH12I	7	V1578I	7	0	1.20E-03	**SILVIO**	oh holder	*	6.86E+00	6.86E+00	7	0
GH12I	7	V1578I	7	0	1.20E-03	SCAR			-1.50E+00	0.00E+00	7	0
G195I	7	V1624I	7	0	1.18E-03	**FAT**	oh holder	*	7.43E+00	7.43E+00	7	0
G195I	7	V1624I	7	0	1.18E-03	ICS			-4.57E+00	0.00E+00	7	3
G123I	7	V2139I	7	0	4.48E-02	**OZ**	s holder	+	3.00E+00	8.29E-02	7	0
G123I	7	V2139I	7	0	4.48E-02	JEFF			2.91E+00	0.00E+00	7	0

Note: TOM took over the harem control from OZ during the period of concentration of female oestrus (second half of the season). He held the harem for one week and consequently achieved a relative high number of matings

Harem SI196

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3858D	7	A090I	7	0	1.85E-03	**FAT**	s holder	*	6.89E+00	4.30E+00	7	0
V3858D	7	A090I	7	0	1.85E-03	ICS			2.59E+00	0.00E+00	7	0
G056I	7	A092D	7	0	2.02E-03	**FAT**	s holder	*	6.78E+00	6.78E+00	7	0
G125I	7	A206D	7	0	4.69E-03	**FAT**	s holder	*	5.55E+00	5.55E+00	7	0
G125I	7	A206D	7	0	4.69E-03	MICO			-2.01E+00	0.00E+00	7	2
G327I	7	R409I	7	0	6.95E-03	MAX	secondary	•	-4.42E-02	0.00E+00	7	2
G327I	7	R409I	7	0	6.95E-03	7			-1.15E-01	0.00E+00	7	0
V3692I	7	R467D	7	0	1.34E-02	**FAT**	s holder	*	5.22E+00	5.22E+00	7	0
V3509D	7	R527I	7	0	8.51E-02	**TOM**	t holder	+	2.27E+00	6.01E-01	7	0
V3509D	7	R527I	7	0	8.51E-02	BLIX			1.67E+00	0.00E+00	7	0
GA07D	7	R612D	7	0	5.16E-03	**FAT**	s holder	*	5.92E+00	5.92E+00	7	0
GA07D	7	R612D	7	0	5.16E-03	NICO			-7.50E+00	0.00E+00	7	3
G032I	7	R678I	7	0	2.64E-02	**7**	secondary	*	2.53E+00	2.22E+00	7	0
G032I	7	R678I	7	0	2.64E-02	GLU			3.13E-01	0.00E+00	7	2
V3598I	7	V0082D	7	0	8.26E-03	**FAT**	s holder	*	5.72E+00	5.72E+00	7	0
V3440I	7	V0087D	7	0	3.23E-03	**FAT**	s holder	*	6.62E+00	2.05E+00	7	0
V3440I	7	V0087D	7	0	3.23E-03	ICS			4.58E+00	0.00E+00	7	0
V3518I	7	V0128I	7	0	2.02E-03	**FAT**	s holder	*	6.68E+00	6.68E+00	7	0
V3943I	7	V0191D	7	0	3.90E-03	**FAT**	s holder	*	6.84E+00	6.84E+00	7	0
V3498I	7	V0269I	7	0	5.39E-03	**FAT**	s holder	*	6.06E+00	1.44E+00	7	0
V3498I	7	V0269I	7	0	5.39E-03	ICS			4.62E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
V3819D	6	V0278D	6	0	2.89E-03	**FAT**	s holder	*	5.93E+00	5.93E+00	7	0
G491I	7	V0362I	7	0	3.23E-03	**FAT**	s holder	*	6.82E+00	6.82E+00	7	0
V3888I	7	V0434I	7	0	2.74E-02	**FAT**	s holder	*	2.76E+00	2.76E+00	7	1
V3945I	7	V0439D	7	0	7.90E-03	**FAT**	s holder	*	5.20E+00	5.20E+00	7	0
G445I	7	V0631D	7	0	1.26E-02	**FAT**	s holder	*	4.53E+00	4.27E+00	7	0
G445I	7	V0631D	7	0	1.26E-02	ICS			2.56E-01	0.00E+00	7	0
G095I	7	V0654D	7	0	1.49E-03	**FAT**	s holder	*	7.21E+00	7.21E+00	7	0
G425I	7	V0680I	7	0	1.33E-03	**FAT**	s holder	*	7.32E+00	7.32E+00	7	0
V3968D	7	V0744D	7	0	7.19E-03	**FAT**	s holder	+	5.49E+00	7.46E-01	7	0
V3968D	7	V0744D	7	0	7.19E-03	ICS			4.74E+00	0.00E+00	7	0
G422I	7	V0754I	7	0	2.20E-03	**FAT**	s holder	*	6.60E+00	1.46E+00	7	0
G422I	7	V0754I	7	0	2.20E-03	ICS			5.14E+00	0.00E+00	7	0
G114I	7	V0907D	7	0	1.11E-03	**FAT**	s holder	*	7.47E+00	7.47E+00	7	0
G114I	7	V0907D	7	0	1.11E-03	ICS			-2.37E-01	0.00E+00	7	1
GA54I	7	A539D	5	1	1.17E-01	**FAT**	s holder	*	4.88E+00	2.60E+00	7	0
GA54I	7	A539D	5	1	1.17E-01	ICS			2.28E+00	0.00E+00	7	0
G324I	7	V0954D	7	0	2.25E-03	**FAT**	s holder	*	6.37E+00	1.44E+00	7	0
G324I	7	V0954D	7	0	2.25E-03	ICS			4.93E+00	0.00E+00	7	0
V3756D	7	V1094I	7	0	1.21E-03	**FAT**	s holder	*	7.34E+00	4.73E+00	7	0
V3756D	7	V1094I	7	0	1.21E-03	ICS			2.62E+00	0.00E+00	7	1
G090I	7	V1745I	7	0	9.67E-03	**FAT**	s holder	*	4.89E+00	3.05E+00	7	0
G090I	7	V1745I	7	0	9.67E-03	69			1.85E+00	0.00E+00	7	0

Harem SM96

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G213I	7	A088D	7	0	5.98E-04	**SILVIO**	s holder	*	2.79E+00	2.79E+00	7	0
G213I	7	A088D	7	0	5.98E-04	TRU			-6.65E-01	0.00E+00	7	0
V3865I	7	A100I	7	0	2.23E-02	**SILVIO**	s holder	*	3.65E+00	2.03E+00	7	0
V3865I	7	A100I	7	0	2.23E-02	SOB			1.62E+00	0.00E+00	7	1
V3776I	7	R629D	7	0	7.54E-03	**SILVIO**	s holder	*	4.90E+00	2.65E+00	7	0
V3776I	7	R629D	7	0	7.54E-03	TONNO			2.25E+00	0.00E+00	7	0
G081I	7	V0095D	7	0	9.96E-03	**SILVIO**	s holder	*	4.33E+00	3.93E+00	7	0
G081I	7	V0095D	7	0	9.96E-03	RON			4.02E-01	0.00E+00	7	1
V3871D	7	V0153D	7	0	1.27E-03	**SILVIO**	s holder	*	6.82E+00	6.82E+00	7	0
V3871D	7	V0153D	7	0	1.27E-03	SOB			-1.18E+00	0.00E+00	7	1
V3829D	7	V0279D	7	0	7.09E-03	**CIC**	secondary	*	6.23E+00	4.28E+00	7	0
V3829D	7	V0279D	7	0	7.09E-03	MO			1.95E+00	0.00E+00	7	1
G042I	7	V0404D	7	0	3.54E-04	MICO	secondary	•	-3.02E-02	0.00E+00	7	2
G042I	7	V0404D	7	0	3.54E-04	ICS			-2.36E+00	0.00E+00	7	1
G120I	7	V0567I	7	0	7.24E-04	**SILVIO**	s holder	*	7.45E+00	6.14E+00	7	0
G120I	7	V0567I	7	0	7.24E-04	TOG			1.31E+00	0.00E+00	7	2
G082I	7	V0632D	7	0	2.98E-03	**SILVIO**	s holder	*	6.13E+00	4.88E+00	7	0
G082I	7	V0632D	7	0	2.98E-03	PAZZO			1.25E+00	0.00E+00	7	0
G441I	7	V0656I	7	0	2.22E-03	**SILVIO**	s holder	*	6.18E+00	5.09E+00	7	0
G441I	7	V0656I	7	0	2.22E-03	SOB			1.09E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
G065I	7	V0684I	7	0	7.02E-03	**SILVIO**	s holder	*	4.99E+00	3.75E+00	7	0
G065I	7	V0684I	7	0	7.02E-03	RON			1.23E+00	0.00E+00	7	0
G156I	7	V0834I	7	0	3.01E-03	**SILVIO**	s holder	*	5.74E+00	4.81E+00	7	0
G156I	7	V0834I	7	0	3.01E-03	PAZZO			9.35E-01	0.00E+00	7	1
G314I	7	V0881D	7	0	1.57E-02	**CIC**	secondary	*	5.64E+00	4.49E+00	7	0
G314I	7	V0881D	7	0	1.57E-02	LEO			1.15E+00	0.00E+00	7	1
G091I	7	V1147D	7	0	1.13E-02	**SILVIO**	s holder	*	4.09E+00	2.05E+00	7	0
G091I	7	V1147D	7	0	1.13E-02	LAMPO			2.03E+00	0.00E+00	7	1
V3964D	7	V1274D	7	0	1.03E-03	**SILVIO**	s holder	*	6.29E+00	4.04E+00	7	0
V3964D	7	V1274D	7	0	1.03E-03	BAU			2.24E+00	0.00E+00	7	1

Harem SF97

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BM45I	7	A327D	7	0	2.38E-04	**PEO**	secondary	*	7.36E+00	7.36E+00	7	0
BM45I	7	A327D	7	0	2.38E-04	GLU			-1.60E+00	0.00E+00	7	1
BB96I	7	R427D	7	0	1.36E-02	**SCAR**	s holder	+	4.65E+00	6.47E-01	7	0
BB96I	7	R427D	7	0	1.36E-02	ICS			4.01E+00	0.00E+00	7	0
BE59I	7	R499D	7	0	5.79E-03	**SCAR**	s holder	*	5.46E+00	5.46E+00	7	0
BE59I	7	R499D	7	0	5.79E-03	TRU			-3.95E+00	0.00E+00	7	1
BH55I	7	R628D	7	0	1.35E-03	**SCAR**	s holder	*	7.43E+00	7.43E+00	7	0
BH55I	7	R628D	7	0	1.35E-03	PICO			-1.25E+01	0.00E+00	7	3
BC94I	7	R660D	7	0	1.56E-03	**SCAR**	s holder	*	7.16E+00	7.16E+00	7	0
BC94I	7	R660D	7	0	1.56E-03	CATO			-5.82E+00	0.00E+00	7	3
BB62I	7	V0090D	7	0	4.05E-03	**SCAR**	s holder	*	6.10E+00	6.10E+00	7	0
BB62I	7	V0090D	7	0	4.05E-03	PICO			-9.35E+00	0.00E+00	7	4
BD59D	7	V0153D	7	0	2.13E-03	**SCAR**	s holder	+	6.83E+00	1.28E+00	7	0
BD59D	7	V0153D	7	0	2.13E-03	ICS			5.54E+00	0.00E+00	7	0
BD76I	7	V0260D	7	0	4.79E-03	**SCAR**	s holder	*	5.89E+00	5.89E+00	7	0
BD76I	7	V0260D	7	0	4.79E-03	PICO			-7.51E+00	0.00E+00	7	3
BB54I	7	V0265I	7	0	8.27E-04	**SCAR**	s holder	*	7.86E+00	7.86E+00	7	0
BB54I	7	V0265I	7	0	8.27E-04	PICO			-1.47E+01	0.00E+00	7	4
BD54I	7	V0268D	7	0	3.04E-03	**SCAR**	s holder	+	6.13E+00	6.09E-01	7	0
BD54I	7	V0268D	7	0	3.04E-03	ICS			5.52E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BD66D	7	V0277I	7	0	1.09E-02	**SCAR**	s holder	*	4.70E+00	4.70E+00	7	0
BD66D	7	V0277I	7	0	1.09E-02	BRUTO			-1.70E+00	0.00E+00	7	1
BF32I	7	V0335D	7	0	6.21E-04	**SCAR**	s holder	*	7.90E+00	4.96E+00	7	0
BF32I	7	V0335D	7	0	6.21E-04	SILVIO			2.94E+00	0.00E+00	7	1
BM53I	7	V0379D	7	0	1.60E-03	**SCAR**	s holder	*	7.13E+00	7.13E+00	7	0
BM53I	7	V0379D	7	0	1.60E-03	PICO			-1.22E+01	0.00E+00	7	5
BF78I	7	V0601I	7	0	1.48E-03	**SCAR**	s holder	*	6.92E+00	6.92E+00	7	0
BF78I	7	V0601I	7	0	1.48E-03	ICCIO			-6.46E+00	0.00E+00	7	2
BL08I	7	V0903I	7	0	5.49E-03	**SCAR**	s holder	*	6.06E+00	5.77E+00	7	0
BL08I	7	V0903I	7	0	5.49E-03	ICS			2.88E-01	0.00E+00	7	0
BI22I	7	V1070D	7	0	3.21E-04	**SCAR**	s holder	*	8.89E+00	8.89E+00	7	0
BI22I	7	V1070D	7	0	3.21E-04	ZOTO			-8.42E+00	0.00E+00	7	1
BB02I	7	V3299D	7	0	7.17E-03	**SCAR**	s holder	*	5.47E+00	4.43E+00	7	0
BB02I	7	V3299D	7	0	7.17E-03	COSO			1.05E+00	0.00E+00	7	0
BB03I	7	V3341I	7	0	3.98E-02	**SCAR**	s holder	+	3.28E+00	1.35E+00	7	0
BB03I	7	V3341I	7	0	3.98E-02	ICS			1.92E+00	0.00E+00	7	0
BB83I	7	V3347I	7	0	4.12E-03	**SCAR**	s holder	*	5.84E+00	4.99E+00	7	0
BB83I	7	V3347I	7	0	4.12E-03	ICS			8.42E-01	0.00E+00	7	0
BD55I	7	V3354D	7	0	6.02E-03	**SCAR**	s holder	*	5.69E+00	5.44E+00	7	0
BD55I	7	V3354D	7	0	6.02E-03	ICS			2.49E-01	0.00E+00	7	0
BD00D	7	V3442D	7	0	1.78E-03	**SCAR**	s holder	*	6.98E+00	6.98E+00	7	0
BD00D	7	V3442D	7	0	1.78E-03	PICO			-1.50E+01	0.00E+00	7	2

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BE06I	7	V3673D	7	0	2.68E-03	**SCAR**	s holder	*	6.61E+00	6.61E+00	7	0
BE06I	7	V3673D	7	0	2.68E-03	PERO			-1.92E+00	0.00E+00	7	1
BC06I	7	V3625I	7	0	4.17E-04	**SCAR**	s holder	*	8.61E+00	8.61E+00	7	0
BC06I	7	V3625I	7	0	4.17E-04	PICO			-1.66E+01	0.00E+00	7	3
BE63I	7	V1274D	7	0	1.87E-03	**SCAR**	s holder	*	6.87E+00	6.87E+00	7	0
BE63I	7	V1274D	7	0	1.87E-03	PICO			-1.29E+01	0.00E+00	7	4
BF50I	7	V0872D	7	0	1.04E-03	**SCAR**	s holder	*	7.53E+00	7.53E+00	7	0
BF50I	7	V0872D	7	0	1.04E-03	NASO			-1.69E+00	0.00E+00	7	1
BL12I	7	V2462D	7	0	2.40E-03	ICS	oh holder	•	-1.94E+00	0.00E+00	7	2
BL12I	7	V2462D	7	0	2.40E-03	TRU			-3.38E+00	0.00E+00	7	1

Harem SI297

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BF57I	7	A345I	7	0	2.23E-02	**TOM**	s holder	*	4.05E+00	4.05E+00	7	0
BF57I	7	A345I	7	0	2.23E-02	PEO			-3.47E+00	0.00E+00	7	2
BJ82I	7	A655I	7	0	9.76E-04	**SCAR**	t holder	*	7.67E+00	7.67E+00	7	0
BJ82I	7	A655I	7	0	9.76E-04	ICS			-3.13E+00	0.00E+00	7	1
BA62I	7	R470D	7	0	4.52E-03	**ICS**	beta	*	5.09E+00	3.36E+00	7	0
BA62I	7	R470D	7	0	4.52E-03	SCAR			1.73E+00	0.00E+00	7	1
BF81I	7	R471D	7	0	1.40E-03	**TOM**	s holder	*	6.95E+00	6.95E+00	7	0
BF81I	7	R471D	7	0	1.40E-03	MUC			-5.03E+00	0.00E+00	7	2
BE40I	7	R498I	7	0	4.26E-03	**TOM**	s holder	*	5.53E+00	5.53E+00	7	0
BE40I	7	R498I	7	0	4.26E-03	MUC			-2.00E+00	0.00E+00	7	2
BA37I	7	V0072D	7	0	4.85E-02	**TOM**	s holder	*	3.58E+00	3.31E+00	7	0
BA37I	7	V0072D	7	0	4.85E-02	KOL			2.72E-01	0.00E+00	7	1
BD77I	7	V0103I	7	0	6.41E-03	**BLO**	secondary	*	6.32E+00	6.32E+00	7	0
BD77I	7	V0103I	7	0	6.41E-03	ROS			-5.54E+00	0.00E+00	7	3
BK76I	7	V0106I	7	0	4.84E-02	**TOM**	s holder	*	3.15E+00	2.97E+00	7	0
BK76I	7	V0106I	7	0	4.84E-02	TONNO			1.75E-01	0.00E+00	7	0
BC84I	7	V0128I	7	0	2.11E-03	**TOM**	s holder	*	6.60E+00	6.60E+00	7	0
BC84I	7	V0128I	7	0	2.11E-03	MUC			-9.97E-01	0.00E+00	7	1
BD99I	7	V0129I	7	0	4.13E-02	**TOM**	s holder	*	3.24E+00	3.24E+00	7	0
BD99I	7	V0129I	7	0	4.13E-02	PERO			-4.61E-01	0.00E+00	7	1

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BG64I	7	V0151I	7	0	2.38E-02	**ICS**	s holder §	+	3.33E+00	1.33E+00	7	0
BG64I	7	V0151I	7	0	2.38E-02	ZOTO			2.01E+00	0.00E+00	7	0
BG06I	7	V0156I	7	0	1.94E-02	**ICS**	s holder	*	3.03E+00	3.03E+00	7	0
BG06I	7	V0156I	7	0	1.94E-02	UBO			-3.42E+00	0.00E+00	7	1
BD20I	7	V0212D	7	0	2.39E-02	**TOM**	s holder	*	3.80E+00	3.80E+00	7	0
BD20I	7	V0212D	7	0	2.39E-02	IGOR			-7.88E-01	0.00E+00	7	0
BE70I	7	V0228I	7	0	2.04E-03	**BOLLO**	secondary	*	6.34E+00	6.34E+00	7	0
BE70I	7	V0228I	7	0	2.04E-03	PICO			-1.55E+00	0.00E+00	7	2
BD17I	7	V0437D	7	0	7.09E-02	**TOM**	s holder	*	2.64E+00	2.64E+00	7	0
BD17I	7	V0437D	7	0	7.09E-02	TONNO			-4.89E-01	0.00E+00	7	1
BG68I	7	V0461I	7	0	1.53E-01	**TOM**	s holder	+	2.10E+00	7.23E-02	7	0
BG68I	7	V0461I	7	0	1.53E-01	858			2.03E+00	0.00E+00	7	0
BF74I	7	V0515D	7	0	2.11E-03	**TOM**	s holder	*	6.51E+00	6.51E+00	7	0
BF74I	7	V0515D	7	0	2.11E-03	MUC			-7.36E-01	0.00E+00	7	2
BJ23I	7	V0648I	7	0	3.18E-02	**TOM**	s holder	*	3.74E+00	3.68E+00	7	0
BJ23I	7	V0648I	7	0	3.18E-02	PERO			5.99E-02	0.00E+00	7	1
BH82I	7	V0657D	7	0	4.09E-03	**TOM**	s holder	*	6.35E+00	6.35E+00	7	0
BH82I	7	V0657D	7	0	4.09E-03	MUC			-1.22E+00	0.00E+00	7	2
BG78I	7	V0822D	7	0	4.03E-02	**TOM**	s holder	*	3.83E+00	3.83E+00	7	0
BG78I	7	V0822D	7	0	4.03E-02	PERO			-2.18E-01	0.00E+00	7	1
BH30I	7	V0834I	7	0	5.34E-03	**TOM**	s holder	*	5.28E+00	5.28E+00	7	0
BH30I	7	V0834I	7	0	5.34E-03	MUC			-1.43E+00	0.00E+00	7	2

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BE72I	7	V0877D	7	0	3.21E-02	**ICS**	s holder §	*	3.37E+00	3.37E+00	7	0
BE72I	7	V0877D	7	0	3.21E-02	MUC			-1.23E+00	0.00E+00	7	1
BE82I	7	V1068D	7	0	1.56E-03	**PAZZO**	secondary	*	6.34E+00	5.08E+00	7	0
BE82I	7	V1068D	7	0	1.56E-03	TESTO			1.26E+00	0.00E+00	7	1
BE86I	7	V2034I	7	0	3.74E-02	**TOM**	s holder	*	3.46E+00	3.46E+00	7	0
BE86I	7	V2034I	7	0	3.74E-02	NOBO			-7.00E-01	0.00E+00	7	1
BG08I	7	V2139I	7	0	3.90E-02	**MUC**	secondary	+	4.05E+00	1.31E+00	7	0
BG08I	7	V2139I	7	0	3.90E-02	***ICS***	*s holder §*		2.75E+00	0.00E+00	7	0
BI15I	7	V2193D	7	0	8.61E-04	**TOM**	s holder	*	7.57E+00	7.57E+00	7	0
BI15I	7	V2193D	7	0	8.61E-04	MUC			-4.89E+00	0.00E+00	7	3
BB57I	7	V3332D	7	0	2.80E-03	**TOM**	s holder	*	6.02E+00	6.02E+00	7	0
BB57I	7	V3332D	7	0	2.80E-03	MUC			-2.87E+00	0.00E+00	7	1
BB01I	7	V3351D	7	0	8.44E-04	**TOM**	s holder	*	7.56E+00	7.56E+00	7	0
BB01I	7	V3351D	7	0	8.44E-04	MUC			-4.49E+00	0.00E+00	7	1
BD86I	7	V3365D	7	0	1.76E-03	**TESTO**	oh holder	*	5.45E+00	3.47E+00	7	0
BD86I	7	V3365D	7	0	1.76E-03	GEO			1.98E+00	0.00E+00	7	0
BC27I	7	V3379I	7	0	1.29E-03	**TESTO**	oh holder	*	5.81E+00	4.51E+00	7	0
BC27I	7	V3379I	7	0	1.29E-03	POP			1.30E+00	0.00E+00	7	1
BA60I	7	V3382D	7	0	2.39E-02	**TOM**	s holder	*	4.02E+00	4.02E+00	7	0
BA60I	7	V3382D	7	0	2.39E-02	PEO			-3.57E+00	0.00E+00	7	2
BD26D	7	V3392D	7	0	2.38E-02	**TOM**	s holder	*	4.55E+00	4.55E+00	7	0
BD26D	7	V3392D	7	0	2.38E-02	858			-5.79E-01	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BD21I	7	V3407D	7	0	2.25E-03	**TOM**	s holder	*	6.47E+00	6.47E+00	7	0
BD21I	7	V3407D	7	0	2.25E-03	PERO			-2.74E+00	0.00E+00	7	1
BC32I	7	V3408D	7	0	1.64E-03	**ICS**	s holder §	*	5.55E+00	4.57E+00	7	0
BC32I	7	V3408D	7	0	1.64E-03	SCAR			9.74E-01	0.00E+00	7	1
BC07I	7	V3432D	7	0	8.65E-04	**TOM**	s holder	*	7.58E+00	7.58E+00	7	0
BC07I	7	V3432D	7	0	8.65E-04	MUC			-5.54E+00	0.00E+00	7	2
BF75I	7	V3617D	7	0	5.93E-02	TONNO	secondary	–	3.02E+00	3.04E-02	7	0
BF75I	7	V3617D	7	0	5.93E-02	***TOM***	*s holder*		2.99E+00	0.00E+00	7	0
BD38I	7	V3639D	7	0	5.48E-03	**TOM**	s holder	*	5.30E+00	5.30E+00	7	0
BD38I	7	V3639D	7	0	5.48E-03	MUC			-1.49E+00	0.00E+00	7	2
BI32I	7	V3658D	7	0	5.78E-03	**ICS**	s holder §	*	4.79E+00	4.05E+00	7	0
BI32I	7	V3658D	7	0	5.78E-03	MUC			7.33E-01	0.00E+00	7	1
BF97I	7	V3681I	7	0	3.05E-03	**SOB**	oh holder	+	3.38E+00	2.63E-01	7	0
BF97I	7	V3681I	7	0	3.05E-03	LEO			3.12E+00	0.00E+00	7	0
BJ36I	7	V3786D	5	0	6.06E-03	**ICS**	s holder §	*	4.78E+00	4.78E+00	7	0
BJ36I	7	V3786D	5	0	6.06E-03	RONF			-3.75E+00	0.00E+00	7	1
BI41D	7	V3847I	7	0	8.46E-03	**ICS**	s holder §	*	4.33E+00	4.33E+00	7	0
BI41D	7	V3847I	7	0	8.46E-03	MUC			-1.29E-01	0.00E+00	7	0
BB53I	7	R419I	7	0	2.90E-02	**TOM**	s holder	*	3.89E+00	3.69E+00	7	0
BB53I	7	R419I	7	0	2.90E-02	PERO			1.94E-01	0.00E+00	7	1
BB61I	7	V0710D	7	0	1.23E-02	**TOM**	s holder	*	5.55E+00	5.55E+00	7	0
BB61I	7	V0710D	7	0	1.23E-02	MUC			-2.42E+00	0.00E+00	7	0

Pup	Pup loci	Known mother	KM loci	P-KM mism	Prob non-exclusion	Candidate father	CF status	Con lev	LOD	Delta	CF loci	P-CF mism
BC90D	7	V0087D	7	0	5.67E-02	**TOM**	s holder	*	3.66E+00	3.66E+00	7	0
BC90D	7	V0087D	7	0	5.67E-02	PERO			-1.27E-01	0.00E+00	7	1
BE12I	7	V0900I	7	0	5.36E-05	**PAZZO**	secondary	*	9.89E+00	9.89E+00	7	0
BE12I	7	V0900I	7	0	5.36E-05	TESTO			-4.59E-01	0.00E+00	7	1
BE28I	7	V0130I	7	0	3.42E-02	**TOM**	s holder	*	3.64E+00	3.35E+00	7	0
BE28I	7	V0130I	7	0	3.42E-02	PERO			2.94E-01	0.00E+00	7	0
BF61I	7	V3704D	7	0	2.27E-03	**TOM**	s holder	*	6.41E+00	6.41E+00	7	0
BF61I	7	V3704D	7	0	2.27E-03	CIT			-3.22E+00	0.00E+00	7	1
BF68I	7	R551I	7	0	1.77E-01	***TOM***	*s holder*	–	1.96E+00	4.44E-02	7	0
BF68I	7	R551I	7	0	1.77E-01	TONNO			1.92E+00	0.00E+00	7	0
BG59I	7	V0654D	7	0	1.94E-03	**TOM**	s holder	*	6.62E+00	6.62E+00	7	0
BG59I	7	V0654D	7	0	1.94E-03	PAZZO			-5.09E-01	0.00E+00	7	2
BH43I	7	V3659D	7	0	1.01E-03	**TOM**	s holder	*	7.28E+00	7.28E+00	7	0
BH43I	7	V3659D	7	0	1.01E-03	MUC			-5.41E+00	0.00E+00	7	2
BJ30I	7	R612D	7	0	3.23E-02	**TOM**	s holder	*	3.50E+00	3.50E+00	7	0
BJ30I	7	R612D	7	0	3.23E-02	858			-1.12E+00	0.00E+00	7	0

Note: TOM was the seasonal holder of SI297. However, the harem split in two different groups almost in the middle of the season: TOM kept the control of the larger group of females while ICS (before Beta male of SI297 held by TOM) became the holder of the smaller group. § indicates that the known mother belonged to the new formed harem, of which ICS was the seasonal holder

APPENDIX C – Behavioural estimates of individual reproductive success

Estimates of seasonal reproductive success in 128 males of Sea Lion Island are shown. Each male was present on the island one to four seasons, from 1995 to 1998. For each male, the age at his first season and the total *ENFI* achieved are reported. Males indicated with an asterisk were those used in the analyses. Data are from this study and from unpublished data from Fabiani, Galimberti and Sanvito.

Male ID	Male age	*ENFI* 1995	*ENFI* 1996	*ENFI* 1997	*ENFI* 1998	TOT *ENFI*
118	AD	1	-	-	-	1
*2C	AD	0	0	-	-	0
*410	SAM2	0	2	-	-	2
*412	SAM2	-	0	0	0	0
*591	AD	0	0	-	-	0
*69	SAM2	0	0	-	-	0
*7	AD	5	1	7	7	20
*858	JUV	-	-	0	-	0
*AKKO	AD	0	41	-	-	41
*ALGO	SAM2	-	-	0	0	0
*ALVI	SAM3	-	0	-	-	0
AND	AD	60	-	-	-	60
*BACO	SAM3	-	0	-	-	0
BAM	SAM4	2	-	-	-	2
*BAU	SAM2	-	0	-	-	0
*BLIX	SAM3	-	0	-	-	0
*BLO	SAM2	-	-	2	0	2
*BLOB	AD	0	40	-	-	40
*BOLLO	SAM2	-	0	0	-	0

Male ID	Male age	*ENFI* 1995	*ENFI* 1996	*ENFI* 1997	*ENFI* 1998	TOT *ENFI*
*BOMBO	SAM1	-	-	0	-	0
BUBU	SAM3	0	-	-	-	0
CARL	SAM3	0	-	-	-	0
*CATO	SAM3	-	0	0	2	2
*CIC	SAM3	0	6	28	24	58
CIRI	JUV	-	0	0	0	0
CIRO	SAM4	0	-	-	-	0
*CIOP	SAM1	-	0	-	-	0
*CIT	JUV	-	-	0	-	0
COC	SAM4	0	0	0	0	0
COLIN	JUV	-	-	0	0	0
*CORO	SAM2	O	0	-	-	0
*COSO	SAM2	-	-	0	-	0
*COTTO	SAM1	-	-	0	-	0
*CUB	SAM3	-	0	-	0	0
DAVE	AD	97	-	-	-	97
*DON	SAM4	0	0	-	-	0
DUE	SAM3	0	-	-	-	0
*EOLO	SAM1	-	-	0	-	0
*ETOS	SAM1	-	0	-	-	0
*FAT	AD	85	52	-	-	137
*FILM	SAM1	-	0	-	-	0
GECO	SAM4	0	-	-	-	0
*GEO	SAM2	0	0	0	9	9
*GIALLO	SAM3	-	-	0	-	0
GILLU	SAM2	-	-	-	0	0
*GLU	SAM4	1	1	58	-	60

Male ID	Male age	*ENFI* 1995	*ENFI* 1996	*ENFI* 1997	*ENFI* 1998	TOT *ENFI*
*GRAT	SAM1	-	-	0	-	0
*GRIG	SAM2	-	0	-	-	0
*ICCIO	SAM3	-	-	0	-	0
*ICS	SAM4	1	5	47	-	53
*IELO	SAM2	0	0	18	-	18
*IGOR	SAM2	0	0	4	-	4
*IPIR	SAM1	-	0	0	-	0
*INGO	SAM1	-	0	-	-	0
*IS	SAM1	-	-	0	-	0
JACK	SAM4	0	-	-	-	0
*JEFF	AD	2	22	7	57	88
KIRK	SAM2	0	-	-	-	0
*KOL	SAM1	-	0	0	3	3
*LAMPO	SAM3	-	0	0	1	1
*LEO	AD	118	125	107	-	350
*LICO	SAM3	0	0	0	0	0
*LOBO	SAM2	-	0	0	-	0
LOGO	SAM4	0	-	-	-	0
*LOLLO	SAM3	-	0	-	-	0
*LUIGI	SAM4	-	-	0	0	0
*LUPO	SAM2	0	0	0	60	60
MAC	AD	6	-	-	-	6
*MAX	SAM4	0	0	-	-	0
*MICO	SAM3	-	0	-	-	0
*MINO	SAM2	-	3	-	-	3
*MIO	SAM1	-	0	0	4	4
*MO	SAM1	-	0	-	-	0

Male ID	Male age	*ENFI* 1995	*ENFI* 1996	*ENFI* 1997	*ENFI* 1998	TOT *ENFI*
*MUC	SAM2	-	-	0	0	0
*NASO	SAM1	-	0	0	2	2
*NICO	SAM4	-	-	0	0	0
*NOBO	SAM2	-	0	2	-	2
NOE	SAM3	2	-	-	-	2
*OVO	AD	10	80	89	78	257
*OZ	AD	13	42	-	-	55
PACO	SAM3	0	-	-	-	0
*PAZZO	SAM3	-	0	0	16	16
*PEO	SAM2	0	0	0	-	0
*PERO	SAM2	-	-	0	0	0
*PICO	SAM1	-	0	0	-	0
PINO	SAM4	0	-	-	-	0
*PIPO	SAM4	-	3	-	-	3
*PONGO	SAM4	-	0	-	-	0
*POP	SAM2	0	0	0	0	0
*PROBO	AD	43	21	0	0	64
PUZ	SAM2	-	-	-	0	0
QUI	SAM2	0	-	-	-	0
RED	AD	34	-	-	-	34
*ROCO	SAM2	-	0	-	0	0
*ROM	SAM1	-	0	0	7	7
*RON	SAM2	-	0	-	-	0
*RONF	SAM4	-	-	0	0	0
*ROS	SAM2	0	1	0	4	5
*SAMO	SAM3	-	-	0	0	0
*SCAR	AD	0	?	64	92	156

Male ID	Male age	*ENFI* 1995	*ENFI* 1996	*ENFI* 1997	*ENFI* 1998	TOT *ENFI*
*SCHIZ	SAM1	-	-	0	-	0
SEI	SAM4	0	-	-	-	0
*SILVIO	AD	-	18	0	-	18
*SOB	SAM4	0	2	22	-	24
*SONNI	SAM1	-	0	0	2	2
STE	SAM4	1	-	-	-	1
*TA	SAM1	-	-	0	0	0
*TAGO	SAM3	-	-	0	6	6
*TEO	SAM1	-	0	-	-	0
*TEPO	SAM3	0	0	8	33	41
*TESTO	SAM2	1	3	19	-	23