Molecular Ecology (1999) **8**, 1417–1429

Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*)

J. WORTHINGTON WILMER,* P. J. ALLEN,† P. P. POMEROY,‡ S. D. TWISS§ and W. AMOS*
*Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK, †BBC Natural History Unit, Broadcasting House, Whiteladies Road, Bristol BS8 2LR, UK, ‡Sea Mammal Research Unit, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, UK, §Department of Biological Sciences, Durham University, Durham, DH1 3LE, UK

Abstract

Microsatellites were used to conduct an extensive analysis of paternity of grey seals from two Scottish breeding colonies at North Rona (n = 1189) and the Isle of May (n = 694), spanning more than a decade. A maximum of 46% of pups at North Rona and 29% of pups at the Isle of May could be allocated a father, even though the majority of candidate males for specific study sites within each colony were believed to have been sampled. Based on the paternities which could be assigned, both colonies showed evidence of reproductive skew, apparently due to the presence of approximately five males who were exceptionally successful. Some males were assigned paternities at least 10 years before, and colleagues 10 years after, being sampled, implying a reproductive lifespan of at least 10 years, and there are indications that the real maximum lies in the range 15–20 years. Male grey seals appear to have at least two breeding strategies they can adopt. On land, some males benefit from a traditionally polygynous system. However, between 50 and 70% of grey seal pups born at a particular colony are not fathered by males who are likely to be sampled by us, implying that these males seldom venture ashore here. We conclude that aquatic mating may play a much larger role in the grey seal than has previously been thought.

Keywords: grey seal, microsatellites, paternity analysis, Pinniped, reproductive success

Received 6 February 1999; revision received 1 April 1999; accepted 16 April 1999

Introduction

Theoretical discussions on the evolution of pinniped polygyny (Bartholomew 1970; Stirling 1975) and sexual dimorphism (reviewed in Ralls (1976)) predict that a species such as the grey seal (*Halichoerus grypus*), which breeds colonially on land and displays marked sexual dimorphism, should have a mating system characterized by a strong degree of polygyny. In such species, male reproductive success is expected to correlate strongly with male competitive ability, which in turn is often correlated with body mass (e.g. Clutton-Brock 1989). Dominant males are able to maximize the duration of tenure on territories or among female aggregations and hence have more opportunities to mate. Although little is known about the intraspecific plasticity of mating systems, we might also expect that the degree of polygyny will be influenced by the degree of aggregation shown by females, and by the local operational sex ratios (OSR) (Emlen & Oring 1977). Both these characteristics may be influenced by environmental conditions and breeding site topographies (Stirling 1975; Emlen & Oring 1977; Alexander *et al*. 1979; Boness & James 1979; Anderson & Fedak 1985; Boness 1991; Twiss *et al*. 1998).

Numerous behavioural studies of grey seals lend support to these predictions, showing a polygynous breeding system. Although the relationship between body mass and male competitive ability is ambiguous in grey seals (Anderson & Fedak 1985; Godsell 1991; Twiss 1991; Tinker *et al*. 1995) and males form only an approximation to a linear dominance hierarchy (Twiss *et al*. 1998), variance

Correspondence: J. Worthington Wilmer. Fax: + 44 (0)1223-336676; E-mail: jmw60@cam.ac.uk

in male mating success is primarily correlated with length of tenure, which is in turn a function of social dominance, as determined by success in aggressive encounters. Dominant males are able to maintain longer tenure at the breeding colony (Anderson *et al*. 1975; Boness & James 1979; Anderson & Harwood 1985; Twiss 1991; Twiss *et al*. 1994).

Observed mating success can provide an acceptable proxy for male reproductive success in some species (e.g. Wainstein *et al*. 1998) and it has been proposed that such measures are acceptable for comparisons between populations (Weatherhead & Boag 1997). However, there is now a growing list of species in which the agreement between observed mating success and the number of genetically assigned paternities is poor (e.g. Birkhead *et al*. 1990; Harris *et al*. 1991; Pemberton *et al*. 1992). The grey seal appears to fall into this second category. In a DNA fingerprinting study of seals breeding on North Rona, Scotland, UK only 20% of pups sampled could be allocated a father, even though most behaviourally dominant males had been sampled. For the small number of males where detailed comparisons could be made between observed mating success and actual reproductive success, relative measures of mating opportunity significantly overestimated the numbers of paternities that could be assigned (Amos *et al*. 1993).

The findings of this preliminary study may in part be reconciled by some degree of partner fidelity (Amos *et al*. 1995). However, the full picture will not be known until a more representative (or complete) sample of both males and pups is obtained. In particular, our previous sampling methods were strongly biased in favour of males who managed to maintain positions in the centre of the colony. Very few samples were taken from males occupying peripheral positions around the colony, males who visited central areas for only very brief periods ('transient' males; Boness & James 1979) or at night, or males who spent most of their time in the water around the breeding colony. Similarly, our original sample of pups was limited to those born to a small number of 'study females'. Because these pups included pairs of full-sibs, the already small sample size was effectively reduced further by nonindependence of paternity.

The study of grey seal breeding behaviour now spans more than a decade and we have expanded our sample number from the previous studies by almost an order of magnitude. New approaches to sampling stimulated by the gaps in the earlier studies have enabled us to sample many nondominant, peripheral males, which were previously assumed to have little or no reproductive success. Such a male sample allows us to examine the hypothesis that the majority of grey seal pups are fathered ashore by males of a much wider range of behavioural classes than predicted by a simple polygynous system. We also include a much broader sample of mother–pup pairs. To facilitate all-against-all paternity exclusions, DNA fingerprinting has been supplanted with microsatellite analysis.

Materials and methods

Study sites

Samples were collected from grey seals at two Scottish breeding colonies, North Rona (59°06′N, 05°50′W) and the Isle of May (56°11′N, 2°33′W) (Fig. 1). North Rona is an isolated rocky island lying 75.5 km north-northwest of Cape Wrath, with an area of 120 ha, rising to 108 m above sea level. The Isle of May lies in the Firth of Forth, 9.5 km from the coast of Fife. The island is 2 km long and 0.5 km wide at its widest point, with cliffs rising 40 m above sea level on the south and west.

The two colonies studied here show quantitatively different topographies (Twiss & Thomas 1998; S. D. Twiss *et al*. submitted). North Rona possesses relatively few access points from the sea and the main breeding area comprises relatively open grassy lawns with many small pools of water. Female dispersion patterns here show aggregation around pools of water and access points, but spread up to 300 m inland. In contrast, the Isle of May has more access points but the interior is composed of more broken rocky terrain with smaller, discrete areas of relatively flat, suitable pupping terrain separated by rocky crags and outcrops. Females are constrained to pup in these smaller areas, causing a greater degree of aggregation (Twiss & Thomas 1998; Pomeroy *et al*. 1999). Sex ratios at the Isle of May are more skewed than those at North Rona (average seasonal sex ratios of 1 male: 14 females on the Isle of May compared with 1 : 6 for North Rona; Twiss & Thomas 1998; Twiss *et al*. 1998). Precise ratios are difficult to calculate because within the 6–8-week breeding season, there is constant turnover of both females, who come ashore for approximately 18–20 days, and males who vary greatly in the amount of time they spend in the colony. The two colonies also differ in the timing of the breeding season by approximately 4 weeks with peak pupping occurring at North Rona around 8 October (Pomeroy *et al*. 1994) and at the Isle of May around 5 November (Hiby *et al*. 1992).

Over the course of the study the annual pup production has varied markedly at the two colonies. At North Rona pup production declined from 1600 to the relatively stable current level of around 1200, while at the Isle of May it has increased from fewer than 30 to 1770 over the last 25 years (Pomeroy *et al*. 1994, 1999). Grey seals tend to use the same areas of the islands for breeding in different years and although local densities may vary, there is evidence that breeding females will not tolerate the presence of others at distances of under

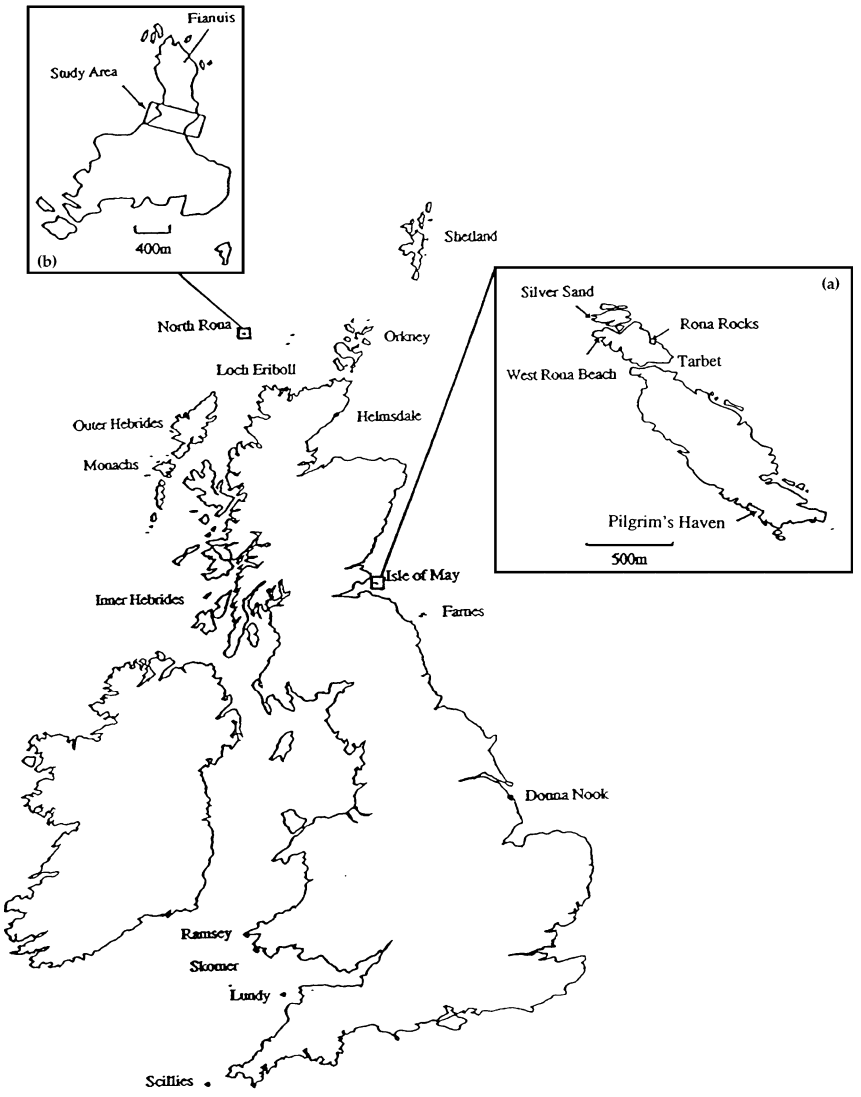

Fig. 1 Map of the British Isles showing the location of the main grey seal breeding sites where behavioural observations and tissue sampling have been conducted on: (a) the Isle of May; and (b) North Rona. On North Rona animals in the study area have been the most intensively studied and sampled. When data are divided into the north/south dichotomy (see text) this division occurs among animals sampled in the north of Fianuis and then Fianuis south and the study area (south). At the Isle of May the most intensive sampling has occurred in the Tarbet, Rona Rocks and West Rona Beach regions.

approximately 3 m (Anderson *et al*. 1975; Pomeroy *et al*. 1994, 1999).

Sampling strategy and sample collection

The samples used in this study were collected from North Rona between 1986 and 1997 and from the Isle of May between 1991 and 1997. Prior to 1994, all sampling of adult animals involved sedation, limiting both the sample size and sampling location. From 1994 onwards, the sampling of adults was achieved by the collection of small (4 mm^2) skin punches using either the remote sampling technique described by Gemmell & Majluf (1997) or with a biopsy head attached to a pole. This change allowed both a large increase in the sample size, and access to males from all parts of the colony. Biopsy punches were cleaned with solutions of chlorhexidine gluconate ('Hibitane', ICI) and ethyl alcohol, and a prophylactic application of antibiotic (oxytetracycline) was applied. Pups were captured and physically restrained, and skin samples were taken from the interdigital webbing of a hind flipper using (piglet) ear notching pliers in a manner similar to that described by Majluf & Goebel (1992).

The skin samples were stored in tubes containing the preservative buffer 20% dimethylsulphoxide (DMSO) saturated with salt (Amos & Hoelzel 1991). In addition to the tissue biopsy samples, 145 DNA samples were available from individuals sampled during the 1986–1989 breeding seasons at North Rona and which had been used in the DNA fingerprinting studies described in Amos *et al*. (1993, 1995). Details of the total sample set are presented in Table 1. The mother–pup data sets of both colonies contain not only unmarked pairs sampled within a year but also the pups of known females sampled over successive years ('study pups'). The fraction of annual pup production which the total 1995 and 1996 sample

Table 1 Summary of numbers of grey seal samples from North Rona and the Isle of May that have been used in microsatellite typing

	North Rona (1986–1989 + 1993–1997)	Isle of May (1991–1997)
Adult males	290	167
Adult females	392	223
Pups with known mothers	507	304
Total	1189	694

Table 2 Polymorphism characteristics, expected heterozygosities and estimates of identity probabilities of microsatellite loci used to type all grey seal individuals at both colonies

Locus	No. of alleles	Size range	North Rona H_E	Isle of May H_E	Prob. identity
Hg3.6	8	85–101	0.781	0.785	0.080
Hg4.2	8	139–165	0.626	0.695	0.179
Hg6.1	6	150–166	0.620	0.630	0.207
Hg6.3	6	219–229	0.786	0.725	0.078
Hg8.9	11	197–217	0.848	0.818	0.040
Hg8.10	10	183–201	0.781	0.779	0.074
Hgdii	8	111–141	0.679	0.720	0.147
SGPv9	7	160–172	0.805	0.803	0.067
SGPv11	8	160–174	0.709	0.720	0.131
Overall	6.9		0.737	0.742	8.8×10^{-10}

represents was 15.1% at North Rona and 6.5% at the Isle of May. All sampling procedures were carried out under Home Office licence.

DNA extraction and microsatellite typing

Total genomic DNA was extracted from the skin biopsy samples either by the method described in Allen *et al*. (1995) or in a 5% Chelex 100 (Bio-Rad) solution with proteinase K (10 mg/mL) and RNase (10 mg/mL) and incubated for 3–4 h at 55 °C. All DNA samples were screened with a panel of nine dinucleotide repeat microsatellite loci comprising seven isolated from grey seals (Allen *et al*. 1995; Hg prefix) and two from the harbour seal, *Phoca vitulina* (Goodman 1997; Pv prefix). A summary of the loci characteristics is presented in Table 2.

Identity checking

All files were checked for duplicate entries in order to identify individuals that may have been sampled more than once, within and/or between years, using a program called IDENTITY (Allen 1995). The probability of identity (Paetkau & Strobeck 1994) was calculated for each locus and across all loci in order to assess the number of identical genotypes that we expected to find by chance. Any duplicates were subsequently excluded from the genotype files prior to the paternity analyses. Checking for genotype identity between years allowed resampling rates to be estimated and by comparing genotype identity among populations we could establish if there were any movements of individuals between colonies. Mismatches in the mother–pup data sets were located manually and double-checked with reference to the original gels. All scoring errors were corrected and all genuine nonfilial mismatches (North Rona, 74 pups; the Isle of May, 57 pups) were excluded from the paternity analyses.

Null alleles

In the previous study of the population genetics of these two grey seal populations, Allen *et al*. (1995) reported that two of the loci (Hg4.2 and Hgdii) at North Rona had genotype frequencies which deviated significantly from Hardy–Weinberg (HW) equilibrium, suggesting the presence of null alleles. Given the greatly expanded sample sizes used in this study, we re-examined the data sets for the presence of null alleles in both sexes using the same iterative program as before (Allen *et al*. 1995). While the program still used deviations from HW to infer the presence of null alleles it also now included a randomization algorithm which determined the 95% confidence intervals.

Paternity analyses

Paternity analyses were performed using two different programs: NEWPAT version 1.6 (witten by Amos) and CERVUS version 1.0 (Marshall *et al*. 1998). NEWPAT version 1.6 is a general paternity analysis program, compiled in TURBO BASIC, which searches for parent–offspring relationships according to user-inputted criteria and then uses a randomization

approach to assess the significance of any matches found. It incorporates the following relevant features. Allele frequencies are derived separately for each of a mother–offspring file and a candidate male file, including best-fit estimates of any possible null alleles. This approach is used because in many mammals, including grey seals, females are strongly philopatric (Greenwood 1980; Pomeroy *et al*. 1994). Therefore, our sample of females may include close relatives, potentially causing slight differences in allele frequencies between the sexes. Such an effect is probably subtle, but could introduce bias into both the randomization significance tests and estimates of relatedness, hence our use of two separately estimated allele frequency distributions.

Paternity matching was conducted after setting three parameters: (a) the maximum number of unscored loci allowed in any one male–offspring comparison; (b) the maximum number of mismatching loci allowed, usually set at 0 = stringent or 1 = relaxed; and (c) the minimum acceptable probability for a match requiring null alleles (all instances where a match could exist by invoking a null allele were considered).

Each male–offspring match was then assessed by randomization. The male-specific allele frequencies were used to draw random alleles, so creating a file of 'pseudomales'. By default, the number of random genotypes created was set at 100 times the size of the male data set. In this way, the number of matches found by randomization equated to the percentage probability that the original data set would yield at least one match by chance alone. To aid with assessment, relatedness values were calculated for all male–offspring matches, following Queller & Goodnight (1989), and pseudomale matches were only accepted if they yielded a relatedness value as large or larger than the original match.

Finally, background levels of paternity assignment were determined by generating 1000 pairs of data files containing pseudo mother–offspring pairs and pseudomales, and paternity testing these, one against the other. From these, the program calculated the average number of males achieving 0, 1, 2, 3, . . . n paternities. Using the estimate of the background level of paternity assignment, the probability of excluding all males was calculated for each colony. NEWPAT version 1.6 is available for distribution: copies and a full description can be obtained by contacting W. Amos (E-mail: w.amos@zoo.cam.ac.uk).

For our analyses, we allowed no mismatches, used a low acceptable probability of null matches ($P = 0.03$) and allowed one unscored locus. A paternity was assigned to a male if: (a) it was the only male found to match a pup; or (b) it was the male with the highest r-value and lowest randomization number among multiple candidates. Occasionally, two males had r-values differing by 0.01 or less. In such cases, each male was assigned half a paternity. Half paternities were allocated to 20 males for 12 pups at North Rona and to four males for two pups at the Isle of May.

CERVUS version 1.0 (Marshall *et al*. 1998) is a simulation program which generates critical log-likelihood scores to assign paternity at a given level of statistical confidence. The simulation again incorporates user-defined input parameters such as the total number of candidate males, the proportion of those males that have been sampled and the frequency of gaps and errors in the genetic data.

CERVUS requires as an input parameter the total number of candidate males. We defined 'candidate males' as adults who came ashore in those areas of the colonies under daily observation and were seen on the colony at some point during the breeding season. In these specific subregions, sampling was most intense, and we estimated that 85% and 80% of candidates were sampled at North Rona and the Isle of May, respectively (P. P. Pomeroy, personal observation). The degree to which these sampling rates should be extrapolated to other areas where sampling was less intensive is uncertain, but we used values of 350 candidates for North Rona and 210 candidates for the Isle of May. Other parameters set for CERVUS were 0.6% and 1% for the proportion of unscored loci and our observed typing error rates of 0.016 and 0.018 per complete genotype (values for North Rona given first). Paternities were assigned at levels of 80% and 95% confidence.

Cross-population paternities

In addition to within-colony analyses, pups from one colony were screened against the males from the other in order to examine whether any of the pups were fathered by males from the other breeding colony. Given the differences in timing of the breeding season between the two colonies, it is unlikely that there would be more than a handful of true cross-colony paternities, and therefore this analysis also provided an estimate of the background level of spurious assignments.

Male reproductive profiles

Our study spans more than a decade, allowing us to examine changes in reproductive success over time. In general, a random sample of males will include some individuals near the start of their reproductive careers and some near the end. Hence, the combined reproductive success of such a group will decline either side of the year in which they were sampled, and the rate of decline will tell us something about average reproductive longevity. More complicated patterns are expected if most males are sampled either early or late in their careers and if reproductive success changes with age.

To examine changes in male reproductive success over time, we classified our paternity data by the relative years

in which the fathers and pups were sampled. Because pups were sampled the year after they were conceived, we defined zero displacement as when a male sampled in year x is compared against pups born in year $x + 1$. Males assigned to pups born in subsequent years were given positive displacement scores, with comparisons involving pups born in years $x + 2$, $x + 3, \ldots x + n$ being assigned displacements of 1, 2, $\ldots n - 1$. Similarly, males assigned to pups born in seasons x, $x - 1, \ldots x - n$ were given displacement scores of –1, –2, $\ldots -n - 1$.

Degree of polygyny

Given the structure of our data set, there is no simple way by which to assess the degree of polygyny. Comparisons within a season are hampered by both the sample of males which is biased towards males in the specific study areas and the small number of paternities which can be assigned. Equally, a summation of success over several seasons is problematic because the zero success class will be artefactually inflated by comparisons between pups and males, who for any of a number of reasons, were not present in the year of conception. As a conservative measure, we therefore restricted our analysis to male–pup combinations where the male was seen as an adult ashore in the year of conception. Using a simple randomization algorithm, we then tested the null hypothesis that all males have equal reproductive success over the entire study period.

Results

Genotype identity and resampling rates

The very low probability of identity (1×10^{-9}; Table 2) indicates that finding a genoptype match by chance using the nine microsatellite loci described here is almost negligible and implies that any identical genotypes found in the data sets are probably a result of resampling events. Within breeding seasons, resampling rates ranged from 0% for North Rona females, 0–1.9% for Isle of May females, 0–5.9% for North Rona males to 0–11.76% for Isle of May males. Resampling individuals within years is not surprising, due to the large number of unmarked individuals being sampled. The greater resampling rates for males presumably reflect the fact that females remain close to their pups. Also, males can range widely over the colony and it was considered preferable to resample a male rather than risk not sampling an individual altogether.

Between years, only 2.2% and 2.9% of females were resampled over consecutive years (1995–1996 only), compared with 3.2% and 8.5% of the males (over all years), both data pairs being for the Isle of May and North Rona, respectively. These rates suggest that the pool of males accessible by our current sampling methods was smaller than the pool of females, a trend which is consistent with colony sex ratio observations.

Two instances in which a pup had returned to its natal colony to breed were detected. At North Rona the male pup born to female R4 in 1989 was sampled as an adult on the colony during the 1995 and 1996 breeding seasons. At the Isle of May, a female pup born to marked female H7 in 1991 was sampled as a breeding female in 1996. Males originally branded G5 and G6 were later rebranded as S9 and Y2, respectively, and these changes were also confirmed by the genotyping. No genotype matches of any kind were found between breeding sites, even though all possible comparisons were made.

Null alleles

The results of the test for the presence of null alleles are presented in Table 3 and indicate that while there were nonzero estimates for males and females at both colonies, none of these was significant. In several instances, slight but significantly negative test values were observed, suggesting a deviation from HW in the direction of heterozygous excess. However, none of these remained significant after Bonferroni correction for multiple tests, suggesting that both North Rona and the Isle of May are in HW equilibrium.

Paternity analyses (CERVUS)

Using the 95% confidence level, CERVUS version 1.0 assigned a paternity to a total of 104 (20.5%) pups on North Rona and to 66 (21.7%) on the Isle of May (Table 4). For both colonies, the percentage of pups for which paternity could be assigned at the 95% confidence level was half that predicted by the program's simulation test and 15–20% lower at the 80% confidence level (Table 4) suggesting that the assumptions required for our assessment of the numbers of candidate males in the system might be incorrect.

Paternity analyses (NEWPAT)

North Rona. A total of 233 paternities were identified at North Rona (Table 5), accounting for 45.9% of pups and including 22 matches previously assigned by DNA fingerprinting (Amos *et al*. 1993). Correcting for the background rate of paternity assignment (0.35 paternities/1000 male–pup comparisons = 51.46 paternities) the total number of paternities that could be allocated is reduced to 181, or 35.7% of all pups. This background error rate translates to exclusion probabilities of 0.899 (the probability of excluding all males in our North Rona data set) and 0.9997 (the probability of excluding any one male). The

Table 3 Estimates of null allele frequencies and 95% confidence intervals (CI) for deviations from Hardy–Weinberg at each locus for males and females at North Rona and the Isle of May, negative values adjusted to zero

	Hg3.6	Hg4.2	Hg6.1	Hg6.3	Hg8.9	Hg8.10	Hgdii	SGPv9	SGPv11
North Rona females									
Nulls	−0.004	−0.021	−0.018	0.002	−0.017	−0.020	−0.006	−0.004	−0.005
Nulls adjusted	0	0	0	0.002	0	0	0	0	0
95% CI	0.018	0.022	0.023	0.019	0.012	0.017	0.021	0.015	0.019
North Rona males									
Nulls	−0.014	0.008	−0.084	−0.008	−0.026	−0.010	−0.004	0.016	−0.012
Nulls adjusted	0	0.008	0	0	0	0	0	0.016	0
95% CI	0.029	0.039	0.044	0.030	0.025	0.030	0.034	0.025	0.045
Isle of May females									
Nulls	−0.020	−0.025	−0.008	0.0003	−0.026	−0.035	0.008	−0.018	0.011
Nulls adjusted	0	0	0	0.0003	0	0	0.008	0	0.011
95% CI	0.021	0.030	0.026	0.024	0.022	0.020	0.023	0.021	0.027
Isle of May males									
Nulls	−0.045	0.040	−0.01	0.007	0.005	−0.037	0.006	−0.016	0.032
Nulls adjusted	0	0.040	0	0.007	0.005	0	0.006	0	0.032
95% CI	0.039	0.047	0.052	0.047	0.036	0.040	0.044	0.036	0.046

Table 4 Critical log likelihood (ΔLOD) scores, predicted and observed number of paternities assigned at both grey seal breeding colonies at varying levels of confidence. Critical ΔLOD scores and the predicted paternity success rates were generated from 10 000 simulations using CERVUS version 1.0 (Marshall *et al*. 1998)

Population	Confidence level	Critical ΔLOD score	Predicted paternities	Paternities allocated
North Rona	95%	1.49	221 (43.6%)	104 (20.5%)
	80%	0.39	401 (79.0%)	322 (63.5%)
Isle of May	95%	1.52	137 (45.1%)	66 (21.7%)
	80%	0.43	233 (76.6%)	176 (57.8%)

Table 5 Paternity assignment among North Rona and Isle of May males both within pup cohorts and over all years

North Rona					Isle of May				
Pup cohort (no. of pups)	No. of paternities/male	No. of males	No. of paternities	Percentage of males	Pup cohort (no. of pups)	No. of paternities/male	No. of males	No. of paternities	Percentage of males
	0	246		84.8		0	147		88.0
Study pups	1	36	35	12.4	Study pups	1	13	12	7.8
(146)	2 +	8	20	6.9	(76)	2 +	7	14	4.2
			55 (37.7%)					26 (34.2%)	
	0	236		81.4		0	148		88.6
1995	1	42	40	14.5	1995	1	15	15	9.0
(136)	2 +	12	27	4.1	(87)	2 +	4	9	2.4
			67 (49.3%)					24 (27.6%)	
	0	207		71.4		0	138		82.6
1996	1	62	58.5	21.4	1996	1	22	21.5	13.2
(225)	2 +	21	52.5	7.2	(141)	2 +	7	14.5	4.2
			111 (49.3%)					36 (25.5%)	
	0	150		51.7		0	114		68.3
All years	1	81	81.5	27.9	All years	1	33	33.5	19.8
(507)	2 +	59	151.5	20.4	(304)	2 +	20	53.5	11.9
			233 (45.9%)					87 (28.6%)	

number of paternities assigned to individual males was unexpectedly low. Over half the males sampled (51.7%) were not assigned any paternities, and a further 27.9% of males were assigned only a single paternity. The remaining males (20.4%) were found to father 65.0% of the pups to whom fathers could be allocated (Table 5), with the maximum number of paternities allocated to any one male over all years being seven. One male was allocated six paternities in a single season.

Isle of May. At the Isle of May, a total of 87 (28.6% of all pups) paternities were assigned (Table 5). After correcting for the background rate of paternity assignment (here, 17.77 paternities) the total number of paternities that could be allocated is reduced to 69, or only 22.7% of all pups. This background error rate translates to a probability of 0.942 for excluding all males in our Isle of May data set and 0.9996 for excluding any one male. An even greater percentage (68.3%) of males than at North Rona were not assigned any paternities and a further 19.8% of males were assigned a maximum of one paternity. The remaining males (11.9%) were linked to 61.5% of the pups to whom paternities could be allocated. The maximum number of paternities allocated to any one male over all years was seven, while within any 1 year no more than three paternities were assigned to one male.

Paternity assignment comparison: CERVUS vs. NEWPAT

Of the 233 paternities that were allocated at North Rona by NEWPAT, 70 (30.0%) were assigned by CERVUS at the 95% confidence level, 76 (32.6%) at the 80% confidence level and 38 (16.3%) were considered to be the most likely, making an overall level of agreement between the two programs of 78.9%. The extent of disagreement for the remaining 49 (21.1%) paternities ranged from the two programs allocating completely different fathers through to simply being in conflict as to which male of a multiple group of candidates was the most likely father. A rather striking example of the former is a paternity allocated by both DNA fingerprinting (Amos *et al*. 1993, 1995) and NEWPAT to one male but assigned at only the 80% confidence level to a completely different male by CERVUS. There were 33 paternities which CERVUS allocated at the 95% confidence level which were not assigned by NEWPAT. These paternity assignments contained mismatches at up to two loci between the pup and candidate male genotypes and/or were incompatible mother–pup–father triads (i.e. where the pup has to inherit the same allele from both parents) at as many as three loci.

For the Isle of May, the overall level of agreement between the two programs was 89.7%, higher than at North Rona. Of the 87 paternities allocated by NEWPAT, 39 (44.8%) were assigned by CERVUS at the 95% confidence level, 29 (33.3%) at the 80% confidence level and 10 (11.5%) were considered the most likely male. The reasons for disagreement over the remaining nine (10.3%) paternities were as before. Twenty-seven paternities were allocated by CERVUS at the 95% confidence level but not by NEWPAT, and again these contained both multilocus mismatches and/or incompatible triads.

Overall agreement between the two programs was remarkably good (approximately 80–90%), with the primary difference being CERVUS's acceptance of paternities which included multilocus mismatches or incompatible mother–pup–father triads. However, because we were fundamentally interested in attempting to account for the shortfall between the numbers of paternities assigned and the number of males sampled we felt it was inappropriate to use a program which required us to estimate, a priori, the numbers of candidate males in the system. Consequently, for further analysis we consider only the output from NEWPAT.

Degree of polygyny

Based only on pups compared against males who were seen or sampled in the same colony in the year of the pup's conception, our simulations allowed us to reject a model in which all males have equal and constant reproductive success at $P = 0.005$ at both colonies. Factors responsible for this skew could include heterogeneity of sampling within the colony, changes in male reproductive success with age and genuine differences in lifetime reproductive success among males.

Geographic variation in paternity assignment

A total of 47 North Rona pups could be assigned to males from the Isle of May, of which 14 had not been previously allocated to any male from North Rona. Among Isle of May pups, 51 could be allocated to North Rona males, of which 27 had not previously been assigned a father from the Isle of May males. There is no evidence that the number of Isle of May pups assigned to North Rona males was greater than would be expected by chance ($\chi^2 = 0.038$, d.f. = 1, ns). However, North Rona paternities assigned to Isle of May males were slightly more than expected by chance ($\chi^2 = 4.07$, d.f. = 1, $P = 0.045$), implying that one or two may be genuine.

At North Rona in particular, sampling rates of males varied considerably between major colony subregions, ranging from perhaps 85% of all males who came ashore for significant periods in the intensively sampled study area in the south part of the island, down to less than 50% in the north. Such heterogeneity might cause large differences in the probability of paternity assignment among

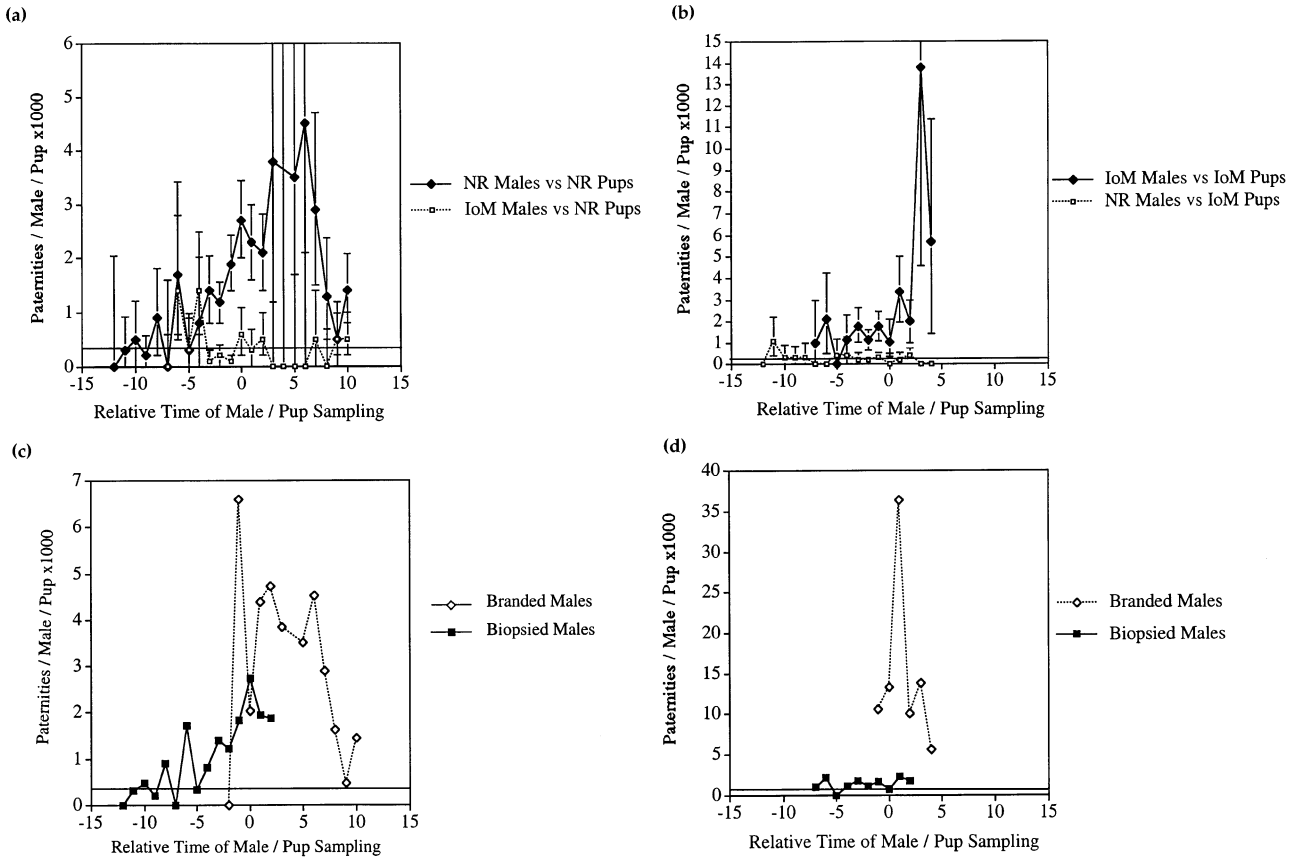

Fig. 2 (a) and (b) present the reproductive profiles averaged over all males at both North Rona and the Isle of May, respectively, and show the error bars (exact binomial 95% confidence intervals) on data points other than zero. Error bars for zero categories have been omitted because where sample sizes are small both negative and positive errors become substantial. For (a) and (b) solid lines and figures represent paternities of males matched against pups from the same colony, while dotted lines and open squares represent the cross-colony paternities. (c) (North Rona) and (d) (Isle of May) show the comparison of male reproductive profiles when partitioned between branded (open figures) and biopsied (solid figures) individuals. The line plotted across the bottom of each graph represents the average background rate of paternity assignment of 0.35 paternities/1000 male–pup comparisons.

pups born in different regions of the colony. However, this appears not to be the case. Paternity assignment rates were virtually identical for pups born in the north (46.0%) and south (45.9%) of the island.

Changes in male reproductive success over time

Figure 2 presents a summary of the paternity analysis, averaged over all comparisons which could be made for each level of displacement between the year a pup was conceived and the year its putative father was sampled. No correction has been made for the presence of spurious paternities due to type I errors, although the average background rate of 0.35 paternities/1000 male–pup comparisons is indicated. At both sites, the rate of intra-colony paternity assignment greatly exceeded both the background level indicated by simulation, and the rate of intercolony paternity assignment.

At North Rona, where both sample size and study period were greatest, a clear trend seems apparent (Fig. 2a). Reproductive success increased steadily from around 10 years before sampling up to 7 years after sampling, followed by a steep decline. However, when the data are partitioned by sampling method, a strong discontinuity is apparent, with branded males on average being two to three times more successful than biopsied males (Fig. 2c). At the Isle of May, the males who were branded show an even greater level of success in terms of paternities per pup–male comparison (Fig. 2d), albeit based on small numbers of both pups and males.

It appears that the five central, dominant males branded at the Isle of May in 1991 were unusually successful (all figures given in paternities/1000 male–pup comparisons, but χ^2 calculations were performed on actual numbers of assigned paternities). On average, they gained significantly more paternities than branded males

on North Rona (Isle of May 11.2, North Rona 1.94; $\chi^2 = 51.3$, 1 d.f., $P \ll 0.001$). Indeed, their success was greater, although not significantly so, than the top five males selected from all those sampled on North Rona (11.2 vs. 6.7, respectively; $\chi^2 = 2.27$, 1 d.f., ns). Interestingly, without the top five males, the average success rate of the remaining North Rona branded males becomes almost identical to those of biopsied males on both North Rona (1.49 vs. 1.47, respectively; $\chi^2 = 0.008$, 1 d.f., ns) and the Isle of May (1.49 vs. 1.44, respectively; $\chi^2 = 0.03$, 1 d.f., ns).

At North Rona, the branded male profile appears to exhibit two large dips, one at 0 and one at –2 (Fig. 2c). These are probably due to a combination of the small number of male–pup comparisons these points represent and any possible disruption caused by capture and branding. In addition, the large dip at –2 may contain an element of observation bias. If the main sampling effort of 1985 picked up the majority of the most dominant males, the subsequent 1987 sampling effort would tend to be enriched for less successful males. However, the primary trend shown by the branded males is a decline in success which seems likely to intercept the background level of paternity assignment due to chance at around +11 years. By implication, even though many branded males were already well established in the colony at the time they were first sampled, most of them continued to contribute paternities for 5–6 years and some continued to breed for up to 11 further years.

The North Rona biopsied males showed lower average rates of success, commensurate with their more diverse and often peripheral sampling locations. It might be expected that many of these males would not have been present in sampling seasons prior to the one in which they were sampled, and they may include males at the very start of their reproductive lifespan. These characteristics appear reflected in the empirical profile, which appears, if anything, to be rising as it crosses the zero line. Extrapolating backwards suggests that the combined reproductive success of the biopsied males falls to background levels at around –10 years, indicating that some of these males were gaining paternities as many as 10 years before they were sampled.

Discussion

To extend previous studies of grey seal breeding behaviour, we have analysed a much larger data set which now includes many more individuals spread over a longer time period whose behaviour represents a much more varied cross-section of males within each colony and, in some cases at least, a high proportion of all males who come ashore during daylight hours. At North Rona, the rate of paternity assignment rose from 20% in the previous study (Amos *et al*. 1993) to a maximum (uncorrected) figure of 46%. However, over half the pups on North Rona and over 70% of the pups sampled on the Isle of May still could not be allocated a father despite the inclusion of four times as many candidate males representing all behavioural categories that came ashore during the breeding season. Some skew in reproductive success among males is evident, with a small number of males enjoying well above-average rates of success. Significant numbers of males remain reproductively active for more than a decade.

Although the rate of paternity assignment remains unexpectedly low, among those paternities that can be allocated, significant male reproductive skew is apparent. The pattern is most striking on the Isle of May, where the average success of the five 1991 branded males is up to 30-fold greater than that of all other males. Part of this dramatic difference may be due to the fact that these five males were sampled in the same region as the study females who bore eight of the 17 pups assigned to these males. However, another factor seems likely to involve topographical differences between the two breeding sites. On the Isle of May, far fewer males are able to maintain central positions among the females in the way they can at North Rona (Twiss & Thomas 1998; Twiss *et al*. 1998). Thus, one explanation for the pattern observed is that a handful of males at each site enjoy elevated success, but on the Isle of May these are the only animals likely to be branded, while at North Rona the top males are 'diluted' by significant numbers of less dominant individuals in amongst the female aggregations.

It remains unclear whether the observed difference in success among males is due simply to differences in age, or actually reflects true variation in lifetime reproductive success. However, long-term observations of individuals suggest that some males do show a progression, beginning with transient status, becoming behaviourally dominant and then declining in success (Twiss 1991; P. P. Pomeroy and S. D. Twiss, personal observation since 1987). Moreover, grey seal males with high mating success tend to be older males (Godsell 1991; although cf. Twiss (1991)) and our branded group is probably on average older than the biopsied group.

We have also shown that males occupying more peripheral positions in the breeding colony gain above-background numbers of paternities, and hence contribute to the genetic composition of the colony. However, the number of paternities assigned to peripheral males still falls well short of the number required to account for all pups born at the colonies. Even though in some areas we have sampled a high proportion of all males who come ashore during daylight hours, including effectively all those who adopt central, long-term positions among the breeding females, we continue to fail to find fathers for 60–80% of pups. The simplest explanation for this deficit,

that most unassigned pups are conceived on land, but outside the areas where male sampling was most intense, appears unlikely because assignment rates do not differ between pups born in areas which differ greatly in the intensity of male sampling, and nowhere do assignment rates exceed 50%.

These observations lend strong support to the notion raised by our earlier studies that the traditional description of the grey seal mating system as terrestrial polygyny, centred around behaviourally dominant males competing to maintain positions within aggregations of females, may be part, but is certainly not the whole story. Instead, more than half of all pups born within a breeding colony are fathered by males who spend little if any time ashore in what were previously considered prime positions among the females, at least during daylight hours. This conclusion is further supported by the inability of CERVUS version 1.0 (Marshall *et al*. 1998) to assign more than half the paternities predicted by its simulations.

There are several possible ways to explain the deficit of paternities. First, breeding occurs in autumn when daylight accounts for only one-third of the diurnal cycle. As yet it is difficult to exclude the possibility that there exists a pool of males who only come ashore at night, and hence who remain unsampled. However, this possibility seems rather unlikely given that nocturnal observations, although very limited, do not detect any increase in activity (Anderson 1978) and that there is no evidence of increased disturbance or relocation of individuals in the morning when daylight observations are resumed (S. D. Twiss, personal observation). In addition, it is difficult to accept that males who are too timid or subordinate to venture on land by day could ever accumulate such a high proportion of paternities.

A second possibility is that some pups are being fathered by males from other colonies. Because all unmarked males are classified as local, this mechanism would have to operate by females visiting other colonies and then returning. Such a pattern seems unlikely for several reasons. Most obviously, behavioural observations show that marked females are strongly philopatric (Pomeroy *et al*. 1994, 1999), although the low between-year resampling rate of females raises the possibility that some unbranded females are less consistent in their pupping patterns. While there is some temporal overlap in breeding seasons at both colonies, females may be physiologically unable to change pupping site, as individuals would potentially have to alter their parturition dates by as much as a month in order to become synchronized with the other colony.

The third possibility is that the traditional model of grey seal breeding behaviour has placed too much emphasis on terrestrial activity, with aquatic matings playing a more important role than previously appreciated. Aquatic mating has been reported at grey seal colonies in Scotland (Hewer 1960; Watkins 1990) and Wales (Davies 1949) and has been observed frequently at the Isle of May (P. J. Allen *et al*., personal observation). Fifteen of the 18 species of the Phocidae (the 'true seals') are thought to mate aquatically (Stirling 1983; Boness 1991; Le Boeuf 1991), although detailed knowledge is limited due to the difficulty in observing reproductive behaviour in the water. In 12 of these 15 species, aquatic mating is associated with the use of ice as the breeding habitat (Boness *et al*. 1993). Given that grey seals have an ancestral association with ice (Davies 1949; McClaren 1960; Bonner 1989), the possibility of aquatic mating should be taken seriously, and we believe this is the most plausible explanation for the shortfall in paternity assignment observed. Coltman *et al*. (1998) in a microsatellite study of harbour seals (*Phoca vitulina*) confirmed the difficulty in assigning paternities in an aquatically mating pinniped, being able to allocate fathers to only a maximum of 30% of pups even at low levels (50%) of statistical confidence.

In discussing aquatic mating, we note that there will be a functional difference between inshore mating, involving copulations occurring within tidal inlets bound by rocky promontories, and offshore mating, where seals mate well away from shore in the open ocean. Any inshore mating would occur in a relatively enclosed space where males may have some degree of control over access to females. Indeed, we have noted that some apparently dominant males at the Isle of May do maintain position in these inlets, and come ashore but rarely. Such males could potentially monopolize mating opportunities. By contrast, offshore mating would leave males with little ability to control access to the females, leaving partner combinations to be determined largely, if not wholly, by female choice.

In assessing the importance of aquatic mating, the precise timing of the female fertile period becomes critical given that at least some females have been recorded as cytologically met-oestrus on land (Pomeroy 1998). Therefore, for aquatic mating to result in successful fertilizations, females would be required to leave the pupping site and enter the water before oestrus has occurred, or that the period during which females are fertile may extend beyond the observed terrestrial receptive stage. Furthermore, multiple terrestrial matings have been reported at rates of 2.5 and 2.9 copulations/female, with a high proportion of females who copulated more than once mating with more than one male (Anderson *et al*. 1975; Boness & James 1979; Twiss 1991), and thus it is possible that some form of sperm competition may be operating in determining paternity.

Despite the great differences between the two classes of male, branded and biopsied, both reveal the presence of at least some males who appear to be reproductively

active for at least 10–12 years around their year of sampling. These results seem reasonable given that they lie within the reported life expectancy for male grey seals of 25 years (Hewer 1964), and are consistent with both the estimate of the age of first breeding for males being around 8 years old (Hewer 1964) and the finding that individual males may use a particular breeding colony for up to 10 years (Twiss 1991). Our finding that a male pup born in 1989 was the probable father of a pup sampled in 1995 suggests that an even earlier onset of reproduction is possible. Field data support this finding, with resident males at North Rona being branded at the known ages of 6 years (one male), 7 years (two males) and 8 years (six males).

A reproductive lifespan of 10 years is probably a minimum estimate for several reasons. First, given the dominant status of the branded males, it seems highly unlikely that all these individuals were marked early in their reproductive lives. Most traditional models of male reproductive behaviour suggest that animals near their peak of success tend to be near the end of their reproductive careers. Thus, while the branded males undoubtedly include some younger animals, there may well be some who were dominant at the outset and who are both currently present and gaining paternities. Similarly, biopsied males show a trend which appears to be increasing as it passes the zero displacement line. If the two profiles are different parts of a single pattern, total reproductive longevity would have to be nearer 20–25 years. However, in the absence of evidence that this is so, our data are best interpreted as showing that at least some male grey seals reproduce for around 12–15 years, and that maximum reproductive longevity may be even greater.

Conclusions

We conclude that traditional models of male grey seal breeding behaviour which focus almost exclusively on terrestrial mating success provide an incomplete picture. Instead, it seems that more than half of all pups born in a colony are fathered, most probably, by males who spend little time ashore. Among males sampled on land, we found some reproductive skew, suggesting a degree of polygyny, and individual males may be reproductively active for periods of 10–15 years.

Acknowledgements

We would like to thank the many people who have been involved during this project in the collection of tissue samples, in particular Sheila Anderson and Simon Moss. We are indebted to all the following people who have assisted in the field over the years: J. and R. Baker, C. Bleay, C. Blomquist, C. Bradshaw, A. Brock, L. Brown, E. Bryan, A. Caudron, S. Enderby, M. Fedak, R. Gadbury, K. Gardiner, L. Grigg, A. Hall, R. Harcourt, E. Harding, B. Herod, A. Jones, M. Jüssi, O. Karlson, O. Keily, J. Lawson, S. Licence, D. Lidgard, G. Liddle, B. McConnell, S. Parlane, C. Pomeroy, F. Praetsch, A. Reid, J. Reilly, M. Biuw, P. Witty. We would also like to thank Scottish National Heritage for granting access to the two colonies. Thanks must also go to L. Bernatchez, D. Coltman and another anonymous reviewer for their very constructive comments on the manuscript. This work forms part of NERC funded core research at the Sea Mammal Research Unit and has been further supported by BBSRC, Royal Society and NERC grants to W. Amos. P. J. Allen was supported by a SERC studentship and S. D. Twiss was supported by a NERC studentship 1987–1989 and a NERC fellowship 1994 to present.

References

Alexander RD, Hoogland JL, Howard RD, Noonan KM, Sherman PW (1979) Sexual dimorphisms and breeding systems in pinnipeds, ungulates, primates and humans. In: (eds Chagnon NA, Irons W) *Evolutionary Biology and Human Social Behaviour: an Anthropological Perspective*, pp. 402–435. Duxbury Press, North Scituate.

Allen PJ (1995) Microsatellite analysis of grey seal (*Halichoerus grypus*) breeding systems. PhD Thesis, University of Cambridge, Cambridge.

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1995) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two Scottish breeding colonies. *Molecular Ecology*, **4**, 653–662.

Amos W, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. *Report of the International Whaling Commission (Special Issue* **13**), 99–103.

Amos W, Twiss SD, Pomeroy PP, Anderson SS (1993) Male mating success and paternity in the grey seal, *Halichoerus grypus*, a study using DNA fingerprinting. *Proceedings of the Royal Society of London Series B*, **252**, 199–207.

Amos B, Twiss S, Pomeroy P, Anderson S (1995) Evidence for mate fidelity in the gray seal. *Science*, **268**, 1897–1898.

Anderson SS (1978) Day and night activity of grey seal bulls. *Mammal Review*, **8**, 43–46.

Anderson SS, Burton RW, Summers CF (1975) Behaviour of grey seals (*Halichoerus grypus*) during a breeding season at North Rona. *Journal of Zoology*, **177**, 179–195.

Anderson SS, Fedak MA (1985) Grey seal males: energetic and behavioural links between size and sexual success. *Animal Behaviour*, **33**, 829–838.

Anderson SS, Harwood J (1985) Time budgets and topography: how energy reserves and terrain dictate the breeding behaviour of grey seals. *Animal Behaviour*, **33**, 1343–1348.

Bartholomew GA (1970) A model for the evolution of pinniped polygyny. *Evolution*, **24**, 546–559.

Birkhead TR, Burke T, Zann R, Hunter FM, Krupa AP (1990) Extra-pair paternity and intraspecific brood parasitism in wild zebra finches *Taeniopygia guttata* revealed by DNA fingerprinting. *Behaviour, Ecology and Sociobiology*, **27**, 315–324.

Boness DJ (1991) Determinants of mating systems in the Otariidae (Pinnipedia). In: (ed. Renouf D) *The Behaviour of Pinnipeds*, pp. 1–44. Chapman & Hall, London.

Boness DJ, Bowen WD, Francis JM (1993) Implications of DNA fingerprinting for mating systems and reproductive strategies of pinnipeds. *Symposia of the Zoological Society of London*, **66**, 61–93.

Boness DJ, James H (1979) Reproductive behaviour of the grey

seal (*Halichoerus grypus*) on Sable Island, Nova Scotia. *Journal of Zoology*, **188**, 477–500.

Bonner WN (1989) *The Natural History of Seals*. Christopher Helm, London.

Clutton-Brock TH (1989) Mammalian mating systems. *Proceedings of the Royal Society of London Series B*, **236**, 339–372.

Coltman DW, Bowen WD, Wright JM (1998) Male mating success in an aquatically mating pinniped, the harbour seal (*Phoca vitulina*), assessed by microsatellite DNA markers. *Molecular Ecology*, **7**, 627–638.

Davies JL (1949) Observations on the grey seal (*Halichoerus grypus*) at Ramsy Island, Pembrokeshire. *Proceedings of the Zoological Society of London*, **119**, 673–692.

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**, 215–223.

Gemmell NJ, Majluf P (1997) Projectile biopsy sampling of fur seals. *Marine Mammal Science*, **13**, 512–516.

Godsell J (1991) The relative influence of age and weight on the reproductive behaviour of male grey seals *Halichoerus grypus*. *Journal of Zoology*, **224**, 537–551.

Goodman SJ (1997) Dinucleotide repeat polymorphisms at seven anonymous microsatellite loci cloned from the European harbour seal (*Phoca vitulina vitulina*). *Animal Genetics*, **28**, 310–311.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.

Harris AS, Young JSF, Wright JM (1991) DNA fingerprinting of harbour seals (*Phoca vitulina concolor*), male mating behaviour may not be a reliable indicator of reproductive success. *Canadian Journal of Zoology*, **69**, 1862–1866.

Hewer HR (1960) Behaviour of the grey seal (*Halichoerus grypus* Fab.) in the breeding season. *Mammalia*, **24**, 400–421.

Hewer HR (1964) The determination of age, sexual maturity, longevity and a life-table in the grey seal (*Halichoerus grypus*). *Proceedings of the Zoological Society of London*, **142**, 593–624.

Hiby AR, Duck CD, Thompson D (1992) Seal stocks in Great Britain: surveys conducted in 1990 and 1991. *NERC News*, January, 30–31.

Le Boeuf BJ (1991) Pinniped mating systems on land, ice and in the water: emphasis on the Phocidae. In: (ed. Renouf D) *Behaviour of Pinnipeds*, pp. 45–65. Chapman & Hall, London.

Majluf P, Goebel ME (1992) The capture and handling of female south-American fur seals and their pups. *Marine Mammal Science*, **8**, 187–190.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

McClaren IA (1960) On the origin of the Caspian and Baikal seals and the paleoclimatological implication. *American Journal of Science*, **258**, 47–65.

Paetkau D, Strobeck C (1994) Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, **3**, 489–495.

Pemberton JM, Albon SD, Guinness FE, Clutton-Brock TH, Dover GA (1992) Behavioural estimates of male mating success tested by DNA fingerprinting in a polygynous mammal. *Behavioural Ecology*, **3**, 66–75.

Pomeroy PP (1999) Breeding behaviour and oestrus in free-ranging grey seals. *European Research on Cetaceans*, **12**, 411–413.

Pomeroy PP, Anderson SS, Twiss SD, McConnell BJ (1994) Dispersion and site fidelity of breeding female grey seals on North Rona, Scotland. *Journal of Zoology*, **233**, 429–447.

Pomeroy PP, Twiss SD, Duck CD (1999) Expansion of a grey seal breeding colony—change in pupping site use at the Isle of May, Scotland. *Journal of Zoology*, in press.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Ralls K (1976) Mammals in which females are larger than males. *Quarterly Review of Biology*, **51**, 245–276.

Stirling I (1975) Factors affecting the evolution of social behaviour in the Pinnipedia. *Rapports et Proces-Verbeaux Des Reunion de Conseil International pour l'Exploration de la Mer*, **169**, 205–212.

Stirling I (1983) The evolution of mating systems in pinnipeds. In: (eds Eisenberg JF, Kleiman DG) *Advances in the Study of Animal Behaviour*, pp. 489–527. American Society of Mammalogists, Shippensburg.

Tinker MT, Kovacs KM, Hammill MO (1995) The reproductive-behaviour and energetics of male gray seals (*Halichoerus grypus*) breeding on a land-fast ice substrate. *Behavioural Ecology and Sociobiology*, **36**, 159–170.

Twiss S (1991) Behavioural and energetic determinants of individual mating success in male grey seals (*Halichoerus grypus*, Fabricius 1791). PhD Thesis, University of Glasgow, Glasgow.

Twiss SD, Anderson SS, Monaghan P (1998) Limited intra-specific variation in male grey seal (*Halichoerus grypus*) dominance relationships in relation to variation in male mating success and female availability. *Journal of Zoology*, **246**, 259–267.

Twiss SD, Pomeroy PP, Anderson SS (1994) Dispersion and site fidelity of breeding male grey seals (*Halichoerus grypus*) on North Rona, Scotland. *Journal of Zoology*, **233**, 683–693.

Twiss SD, Thomas CJ (1998) Fine scale topographical influences on environmental potential for polygamy (EPP) and male reproductive success in grey seals. In: *The Proceedings of the World Marine Mammal Science Conference*, p. 137. Monaco.

Wainstein M, Le Boeuf BJ, Amos W, Gemmell N, Campagna C, Oritz CL (1998) Mating success and paternity in elephant seals. In: *The Proceedings of the World Marine Mammal Science Conference*, pp. 143–144. Monaco.

Watkins JF (1990) Observations of an aquatic grey seal (*Halichoerus grypus*) mating. *Journal of Zoology*, **222**, 677–680.

Weatherhead PJ, Boag PT (1997) Genetic estimates of annual and lifetime reproductive success in male red-winged blackbirds. *Ecology*, **78**, 884–896.

This paper is part of the work resulting from a long-term collaborative project investigating the breeding behaviour and ecology of Scottish grey seals. Jessica Worthington Wilmer is a postdoc working with Bill Amos at the University of Cambridge where the microsatellite analysis of grey seal mating systems begun by Penny Allen during her PhD studies is continuing. Paddy Pomeroy at NERC's Sea Mammal Research Unit, St. Andrews and Sean Twiss at Durham University are seal biologists who, since 1987, have been studying many different aspects of the behavioural and reproductive ecology of grey seals.
