Molecular Ecology (1998) **7**, 627–638

Male mating success in an aquatically mating pinniped, the harbour seal (*Phoca vitulina*), assessed by microsatellite DNA markers

D. W. COLTMAN,*‡ W. D. BOWEN,† and J. M. WRIGHT*
Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada, †Marine Fish Division, Department of Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth Nova Scotia, Canada.

Abstract

Similar to many other pinniped species, harbour seals (*Phoca vitulina*) mate exclusively at sea. Here we present the first attempt to measure male mating success in an aquatically mating pinniped. Male mating success was estimated by paternity analysis in two cohorts of pups born at Sable Island, Nova Scotia, Canada, using microsatellite DNA markers. The genotypes of 275 pups born in 1994 and 1995 were compared to those of 90 candidate males at six microsatellite loci using a likelihood approach to resolve paternity. Paternity could be assigned for two, 22, 40 and 85 pups at confidence levels of 95, 80, 65 and 50%, respectively. Most successful males were assigned the paternity of a single offspring, suggesting a low variance in male mating success relative to most pinniped species. The proportion of paternal half sibs within cohorts and between maternally related sibs estimated by maximum likelihood were not significantly different from zero. It is thus unlikely that most offspring were sired by a small number of highly successful unsampled males, and that female harbour seals do not usually exhibit fidelity to the same male in sequential breeding seasons. A low level of polygyny in Sable Island harbour seals is consistent with predictions based on their breeding ecology, as females are highly mobile and widely dispersed in the aquatic mating environment at Sable Island.

Keywords: harbour seal, mating success, microsatellite, paternity analysis, *Phoca vitulina*, pinniped

Received 1 August 1997; revision received 27 October 1997; accepted 28 November 1997

Introduction

Studies of the variance in reproductive success of both sexes are critical to understanding demography, genetic structure and processes of selection in natural populations. In most mammals, males maximize their fitness by siring as many offspring as possible, provided that females do not require male assistance to rear offspring (Trivers 1972; Emlen & Oring 1977). Males therefore compete intensely for mates and mating success may vary greatly among males. The extent to which male mating success will vary in a population is subject to the interplay of ecological and phylogenetic factors which determine the distribution and economic defensibility of oestrus females in time and space (Emlen & Oring 1977).

Correspondence: D. W. Coltman. ‡Institute of Cell, Animal and Population Biology, Edinburgh University, Ashworth Laboratories, West Mains Road, Edinburgh, EH9 3JT, UK. E-mail: David.Coltman@ed.ac.uk

In pinnipeds, morphological adaptations to foraging at sea, coupled with the need to give birth and nurse on shore, limit the spatial distribution of breeding females to locations which are safe from terrestrial predators (Bartholomew 1970). Most pinniped species breed seasonally; therefore this spatial and temporal clustering of breeding females favours the evolution of polygynous mating systems and high variance in male mating success. There is, however, a wide range in the form and degree of polygyny evident among pinnipeds which is partly dependent on the specific features of their breeding ecology (Stirling 1983; Boness 1991; Boness *et al*. 1993). Pinnipeds thus provide an opportunity to study the influence of ecological variation on mating systems, particularly due to their considerable diversity of mating habitats.

All pinnipeds come ashore to give birth and nurse their pups, and approximately 50% of the extant species actually mate on land. However, two otariid species and 15

species of phocid seal, representing almost all the family Phocidae (the 'true seals') mate aquatically (Stirling 1983; Boness 1991; Le Boeuf 1991). Few studies have attempted to measure male mating success in these species due the inherent difficulties in observing behaviour. This lack of information has been identified as a major gap in our knowledge of pinniped mating systems (Le Boeuf 1991; Boness 1991). It is generally thought that the variance in male mating success should be lower when copulation and courtship occur in the water, as males are less able to control the access of receptive females to other mates in a three-dimensional environment, and females are considerably more mobile in the water than on land (Bartholomew 1970; Stirling 1983; Boness *et al*. 1993).

Studies of male mating success in pinnipeds have mostly been limited to terrestrially breeding species through observing associations between individuals during the breeding season (Boness *et al*. 1993). Studies have shown that a few dominant males which can successfully defend resources that attract females, such as beach space within a colony (territory or resource defence polygyny), or aggregations of females themselves (harem or female defence polygyny) may copulate with numerous females (e.g. northern fur seals, *Callorhinus ursinus*, Bartholomew & Hoel 1953; northern elephant seals, *Mirounga angustirostris*, Le Boeuf 1974). As a result, the variance in male reproductive success in terrestrially mating species is thought to be high, and these species are generally considered to be polygynous (Stirling 1983; Boness 1991; Boness *et al*. 1993). However, only Amos *et al*. (1993) have employed genetic methods to verify mating success estimates based on observed copulations in a pinniped. Observations may be biased estimators of male mating success as it may be inaccurate to assume that females only mate with dominant males. Peripheral males may employ less visible sneaker strategies, or mate with females in the water as they depart from the colony, thus leading to an overestimate of the variance in male mating success (Boness *et al*. 1993). In grey seals, Amos *et al*. (1993) found the most-likely candidate male, based on behavioural data, was often not the genetic father. In a subsequent analysis, Amos *et al*. (1995) genetically identified a larger than expected number of full sibs among pups with undetermined paternity, and suggested that mate fidelity and polygyny operate simultaneously at North Rona.

The harbour seal, *Phoca vitulina concolour*, is a slightly dimorphic, relatively small-bodied pinniped (McLaren 1993) which breeds on isolated islands and inlets along the eastern coast of North America (Boulva & McLaren 1979). Harbour seals mate aquatically; however, relatively little is known about male mating success. Each year, females come ashore, give birth and nurse their pups during a relatively short breeding season (4–6 weeks). Mating probably occurs near the time of weaning (Boulva & McLaren 1979; Thompson 1988). Receptive females are therefore clustered geographically and temporally on a scale such that polygyny is possible. At the same time, mating occurs at sea and size dimorphism is weak: characteristics which are more likely to be associated with reduced polygyny relative to other pinnipeds (Stirling 1983).

We sought to estimate the variance in male mating success by determining the paternity of pups born in 1994 and 1995 at Sable Island using single-locus microsatellite markers. We hypothesized that the variance in mating success among males would be relatively low compared to terrestrially mating pinniped species, as male harbour seals may be less able to monopolise the access to, and movements of, receptive females in the aquatic environment. At Sable Island, females have unlimited access to the sea and they are fairly widely distributed along the region of the north beach which is used for pupping (Godsell 1988; Walker & Bowen 1993). Therefore it may be difficult for individual males to secure a large number of mates in a single season.

As the results of paternity analysis may be biased by the selection of males which we were able to sample, we also analysed the distribution of paternally derived alleles shared among pups within each cohort to estimate the proportion of paternal half sibs. This provides a separate assessment of the degree of polygyny in the population which is independent of the biases which may result from sampling males, as we ought to expect a significant proportion of paternal half sibs to occur within cohorts in a small population if mating success is biased towards a small number of males. This approach was also used to estimate the frequency of full sibs born to females sampled in consecutive years, to determine the extent to which females may mate with the same male in subsequent seasons (mate fidelity), as found in grey seals at North Rona, Scotland by Amos *et al*. (1995).

Materials and methods

Study site and sampling strategy

Sampling was conducted during the breeding seasons (mid-May to the end of June) of 1993, 1994 and 1995 on the north beach of Sable Island (43°55'N; 60°00'W), a partially vegetated sandbar 40 km in length, 160 km east of Nova Scotia. Between 200 and 400 harbour seal females gave birth on both the north and south beaches of Sable Island during each year of the study. Our efforts were focused on a 20-km-long section of the north beach where most pups are born. Most harbour seals which haul-out during the breeding season along this section of beach do not move to the south side of the island within the breeding season

(Godsell 1988; W. D. Bowen, unpublished data). During 1993 and 1994 many males were individually marked with fluorescent paint to facilitate identification for other concurrent studies. Resightings of these animals during periodic population censuses were used to estimate the effective number of breeding males in the population for the paternity analysis.

Sample collection and template DNA extraction

Samples from pups were taken during tagging of all harbour seal pups born on the north beach of Sable Island in 1994 and 1995. Whole cohort tagging of harbour seal pups has taken place at Sable Island since the late 1970s (W. D. Bowen, unpublished data). A 6 mm punch was used to create a hole in the webbing of the hindflipper prior to insertion of an individual-specific permanent tag (Dalton Industries) and the disk of skin and underlying tissue was stored in a 5 mL plastic tube containing a preservative solution of saturated NaCl/20% dimethyl sulphoxide (w/v). We attempted to capture mothers simultaneously when handling pups for tagging. A skin sample was similarly taken from the mother's hindflipper, and from every adult male handled and tagged during concurrent behavioural and energetic studies from 1993 to 1994 (i.e. Coltman *et al*. 1997, 1998).

Skin samples were stored at 4°C or – 20°C from the day of collection until extraction. Template DNA was extracted from skin by incubating 5 mg in 200 µL of 5% Instagene DNA Purification Matrix (Bio-Rad) overnight at 60°C in a 1.5 mL microfuge tube, then immersing the tube in a boiling-water bath for 8 min. A volume of 2 µl of the supernatant was routinely used as the template for PCR amplification.

Characterization of microsatellite loci

Six microsatellite loci were employed in paternity analysis (Table 1). PCR amplifications were performed as described in Coltman *et al*. (1996). Observed microsatellite genotype frequencies of adult males and females at each locus were tested for homogeneity by G tests prior to estimating population allele frequencies. For all locus-specific tests, the level of significance was adjusted by Sidak's multiplicative inequality to give an overall significance of $P < 0.05$ (Weir 1990; Sokol & Rohlf 1995). Rare genotypes ($N < 5$) were pooled to avoid bias caused by low expected frequencies. Population allele frequencies were then estimated from the pooled adult genotypes. The variance and 95% confidence limits of allele frequencies were calculated using Weir's (1990) equations for within-population variance.

Allelic disequilibrium was tested by investigating deviation from Hardy–Weinberg equilibrium (HWE) for each locus and deviation from expected frequencies by G tests. Rare allele frequencies were pooled to avoid bias caused by low expected frequencies. Tests for genotypic disequilibrium were calculated for pairs of loci using the pooled adult genotype data in GENEPOP version 1.2 (Raymond & Rousset 1995) which tests for linkage between pairs of loci according to Weir (1990). Sidak's multiplicative inequality was used to adjust the critical level of significance to account for the large number of tests made.

Paternity analysis

The probability of exclusion for each pup j was calculated across all 6 loci as:

$$P(ex)_j = \Pi_k (1 - P(ex)_k) \quad (1)$$

where $P(ex)_k$ represents the probability of exclusion at the kth locus. This was estimated as the sum of the expected frequencies of all paternal genotypes which could not have produced the offspring genotype given the maternal genotype. Exclusionary criteria alone were not considered to be indicators of paternity as this offers no help when multiple males are not excluded as the father of a given offspring, and there is no provision to account for unsampled males. For these reasons we adopted a likelihood approach to paternity assignment.

Formulae derived by Thompson (1976) and first used by Meagher (1986) in an analysis of paternity in a natural population were used to calculate log-likelihood ratios for paternity analysis. Assuming mating is random and that the mother's genotype is known, the likelihood ratio for each male on each pup was calculated as the ratio of the likelihood that the mother and alleged father are the parents of the pup ($L(H_1)$) to the likelihood that the mother is the parent and the father is a randomly chosen individual from the population ($L(H_0)$):

$$L(H_1)/L(H_0) = \frac{P(g_p|g_m,g_a).P(g_m).P(g_a)}{P(g_p|g_m).P(g_m).P(g_a)} \quad (2)$$

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

$$L(H_1)/L(H_0) = \frac{P(g_p|g_m,ga)}{P(g_p|g_m)} \quad (3)$$

and thus represents how much more likely it is that a given male passed his genes to the pup than a randomly selected male. Likelihood ratios were calculated for each male at each locus and the natural logarithm of the product across all six loci was taken. This is called the LOD score (Meagher 1986). The male with the highest LOD score for a given pup was considered to be the most-likely sire for that pup.

Inferences about paternity were made according to the magnitude of the difference between the LOD of the

Table 1 Observed heterozygosities and allele frequencies of microsatellite loci used in paternity analysis of harbour seals at Sable Island, Nova Scotia

Locus*	*N*†	*h*‡	*G* (d.f.)§	*P*	Allele size (bp)	Frequency (95% CI)
β-globin	284	0.73	0.95 (10)	ns	320	0.354 (0.038)
					315	0.097 (0.024)
					310	0.366 (0.039)
					305	0.083 (0.022)
					300	0.100 (0.026)
Hg 8.10	286	0.58	1.46 (2)	ns	165	0.474 (0.039)
					163	0.502 (0.039)
					161	0.007 (0.007)
					159	0.007 (0.007)
					157	0.010 (0.008)
Pvc 19	284	0.46	0.78 (2)	ns	108	0.311 (0.037)
					100	0.689 (0.037)
Pvc 43	270	0.54	0.81 (4)	ns	100	0.517 (0.042)
					98	0.431 (0.042)
					96	0.009 (0.009)
					92	0.043 (0.017)
SGPV 3	271	0.82	2.87 (9)	ns	301	0.002 (0.004)
					281	0.013 (0.010)
					279	0.015 (0.010)
					277	0.015 (0.010)
					275	0.009 (0.010)
					273	0.031 (0.016)
					271	0.011 (0.009)
					269	0.042 (0.017)
					268	0.002 (0.004)
					267	0.017 (0.012)
					265	0.044 (0.018)
					264	0.013 (0.010)
					263	0.109 (0.027)
					262	0.065 (0.023)
					261	0.175 (0.035)
					259	0.113 (0.030)
					257	0.044 (0.019)
					255	0.223 (0.043)
					253	0.007 (0.007)
					251	0.026 (0.014)
					249	0.024 (0.013)
SGPV 11	286	0.43	1.32 (7)	ns	158	0.012 (0.009)
					156	0.752 (0.034)
					152	0.029 (0.014)
					150	0.164 (0.029)
					148	0.042 (0.016)

*Sources of loci (GenBank Accession number) and annealing temperatures (T_A): β-globin [U91911] (R. Slade, unpublished data), T_A 56 °C; H.g. 8.10 [G02093] (Allen *et al*. 1996), T_A 58 °C; *Pvc* 19 [L40989] (Coltman *et al*. 1996), T_A 58 °C; *Pvc* 43 [U94900] (D. W. Coltman, unpublished data), T_A 53 °C; SGPV 3 [U65442], T_A 53 °C and SGPV 11 [U65444], T_A 56 °C (Goodman 1997).

†Number of adult male and female samples typed.

‡Observed frequency of heterozygotes.

§Reduced degrees of freedom for *G* tests of allelic disequilibrium reflect pooling of genotypes with low expected frequencies.

most-likely and the next-most-likely sire (ΔLOD). This approach assumes that all genotypes have been determined without error, such that a mismatch between a male and a pup at any one or more loci implies a paternity exclusion. Genetic data are rarely perfect, however, and inaccuracies may occur for a variety of reasons, including mutation, which may lead to the false exclusion of a true sire. For this reason, we applied a modified version of eqn 2 derived by Marshall *et al*. (1998) which uses an error rate to account for the possibility that one or more of the mother-pup-alleged sire's genotypes has been incorrectly scored. To assign paternity, we used a paternity simulation program (CERVUS) to predict critical ΔLOD scores to assign paternity at a given level of statistical confidence (Marshall *et al*. 1998) with the following input parameters. The total number of candidate males (180) was estimated from field observations based on the frequency of sightings of marked males hauled out during periodic censuses of the total north beach population during other concurrent research. We estimated the proportion of candidate males sampled (0.5) as the ratio of the total number of samples from males included in the paternity analysis to the total number of candidate males. Approximately 4% of genotypes were missing. We estimated the rate of typing error (0.005) from the frequency of mother–pup mismatches. Paternities were assigned at levels of 50, 65, 80 and 95% confidence.

To gauge the resolution of our genetic markers, we simulated datasets consisting of 90 males and 180 females and the offspring produced under varying mating regimes representing increasing levels of reproductive skew. Parental genotypes were created by randomly sampling population allele frequencies (Table 1). Ninety offspring genotypes were then generated assuming that successful males sired a mean of one, two, five or 10 offspring, and the remaining 90 offspring were given paternal alleles at random, under the assumption that the unsampled males sired the remaining 50% of the offspring. Paternity analysis was then performed, and progeny assigned to males at confidence levels of 50, 65 and 80%. Ten simulations for each level of reproductive skew were performed. The standardized variances in the number of paternities were calculated for each simulation and the means compared to estimate the degree of reproductive skew which can be detected using this marker system.

Estimation of the frequency of paternal half sibs within cohorts and full sibs between cohorts

The frequencies of paternal half sibs occurring within cohorts, and between years born to the same female, were estimated by comparing the observed levels of paternally derived alleles shared between pups to levels of allele sharing expected between paternally unrelated and paternally related individuals. For all pups of known maternity, the paternal contribution was deduced by subtracting the alleles which were inherited maternally. Within each cohort, the paternal contribution at each locus was compared between all pups in a matrix consisting of all pairwise comparisons, with half alleles assigned in cases where the paternal contribution was ambiguous. The average level of paternal allele sharing between pups was calculated for each locus, and across all six loci. Similarly, the paternal contributions to pups born to the same female in consecutive years were compared to estimate the proportion of paternal half sibs born to the same female (full sibs).

Paternally unrelated pups are expected to share paternally derived alleles at a given locus with a probability of G, the population gene identity, where:

$$G = \sum_i p_i^2 \quad (4)$$

where p_i represents the frequency of the ith allele at a given locus. This corresponds to the probability of drawing the same allele twice from the population assuming unlinked loci and random mating. Paternal half sibs are thus expected to share paternally derived alleles with probability G_p:

$$G_p = 0.5\,(1 + G) \quad (5)$$

(Amos *et al*. 1995). G resembles a binomially distributed statistic with $p = G$ and $q = 1 - G$, and $k = 1$. Therefore the standard errors of G and G_p for the population were estimated as:

$$\mathrm{SE} = [G \times (1-G)]^{0.5}/N^{0.5} \quad (6)$$

The 95% confidence limits were then taken as:

$$95\%\ \mathrm{CL} = 1.96 \times \mathrm{SE} \quad (7)$$

We calculated the probabilities of observing all possible proportions of paternal half sibs of all pups within cohorts, and between years of pups born to the same female, by binomial expansion for each locus. Probabilities from each locus were then combined into a single log-likelihood curve to estimate the maximum likelihood proportion of paternal sibs.

Results

Sample collection

Skin samples were taken from 90 males between 1993 and 1995. The genotypes of all sampled males were compared to 144 pups sampled in the 1994 cohort and 131 pups sampled in the 1995 cohort. The maternal parent was also captured and sampled for 94.5% of the sampled pups. Sixty-three pups were known to have been born to the same female in consecutive years.

Characterization of microsatellite loci

Observed genotype frequencies did not differ significantly between adult males and females at the microsatellite loci β-globin, H.g. 8.10, *Pvc* 19, *Pvc* 43 and SGPV 11 ($P > 0.05$ for each, data not shown). Due to the large number of different genotypes observed at the locus SGPV 3 ($N = 72$), allele frequencies rather than genotype frequencies were compared between males and females, and they did not differ significantly ($P > 0.05$, data not shown). Genotypic data from all sampled adults were therefore pooled to estimate population allele frequencies (Table 1). Observed heterozygosities ranged from 0.43 to 0.82 (Table 1). There was no evidence of allelic disequilibrium at any locus (Table 1), nor was there evidence of significant genotypic disequilibrium between any pair of loci (following the application of Sidak's multiplicative inequality, $P > 0.0034$ for each comparison, data not shown).

Mismatches occurred between mother and pup genotypes at a single locus eight times. Six of these mismatches were due to gel-reading error and were corrected. However, two mismatches involved differences between the closest maternal and pup alleles by a single repeat unit at the locus SGPV 3, suggesting the transmission of an allele with a mutation of a single repeat unit. No mismatches occurred in the form of the mother and offspring appearing homozygous for different alleles, which would have suggested the transmission of a null allele. The incidence of detectable mismatches was therefore 0.26% (eight in 3040 mother–offspring single locus comparisons). We therefore set the error rate to 0.5% for the likelihood calculations and for the paternity simulation, given that some typing errors or mutations are likely to be undetected.

Paternity analysis

The mean probability of producing a pup's genotype from a mating with a randomly chosen male (denominator of eqn 2) was 0.0019 ± 0.0002 ($N = 144$) and 0.0027 ± 0.0003 ($N = 131$) in the 1994 and 1995 cohorts. The average probability of exclusion was 0.957 ± 0.005 (range 0.309 – 0.999) and 0.940 ± 0.006 (range 0.611–0.999) in the 1994 and 1995 cohorts ($N = 144$ and 131, respectively). If no undetected typing errors occurred, all but one male would have been considered excluded as the possible sire for 21 pups in 1994 cohort (14.6%) and for 14 pups in the 1995 cohort (10.7% of all cases).

Using LOD formulae incorporating typing errors, ΔLOD scores were skewed to the right in both 1994 and 1995 (Fig. 1). The median ΔLOD did not differ significantly between years (0.50 and 0.44, $P > 0.05$, Mann–Whitney *U*-test). Critical ΔLOD values predicted by the CERVUS simulation are also illustrated in Fig. 1 for paternity assignment at confidence levels of 50, 65, 80 and 95%. Paternity could be assigned to between two and 85 pups, depending on the degree of statistical confidence accepted for paternity assignment (Table 2). The percentage of pups for which paternity could be assigned was similar to the success rate predicted by the paternity simulation (Table 2). Examples of the distributions of LOD scores for all sampled males are illustrated for typical paternities assigned at a minimum of 95, 80, 65 and 50% confidence in Fig. 2.

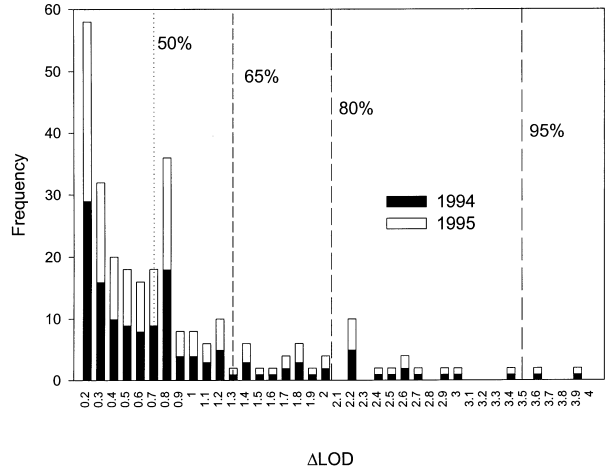

Fig. 1 Distribution of ΔLOD scores of most-likely male candidates in the 1994 and 1995 cohorts. Dotted lines denote predicted critical ΔLOD for 95, 80, 65 and 50% confidence levels predicted by the CERVUS simulation (Marshall *et al*. 1998).

The number of paternities assigned to males was not significantly correlated between cohort years (Spearman rank correlation coefficients, 50% confidence: $r_{90} = 0.04$; 65% confidence: $r_{90} = 0.08$; 80% confidence: $r_{90} = 0.17$) and was unbiased with respect to the number of loci at which individuals were homozygous, as there was no significant difference between the median number of paternities assigned to males with four or more, or those with fewer

Table 2 Critical ΔLOD, predicted and observed number of paternities assigned at varying levels of confidence. Critical ΔLOD and the predicted paternity success rates were generated from 10000 paternity simulations using the CERVUS program (Marshall *et al*. 1998)

Confidence level	Critical ΔLOD	Predicted paternities	Paternities assigned
95%	3.46	2.0%	2 (0.7%)
80%	2.04	6.7%	22 (8.0%)
65%	1.32	16.9%	40 (14.5%)
50%	0.72	31.8%	85 (30.9%)

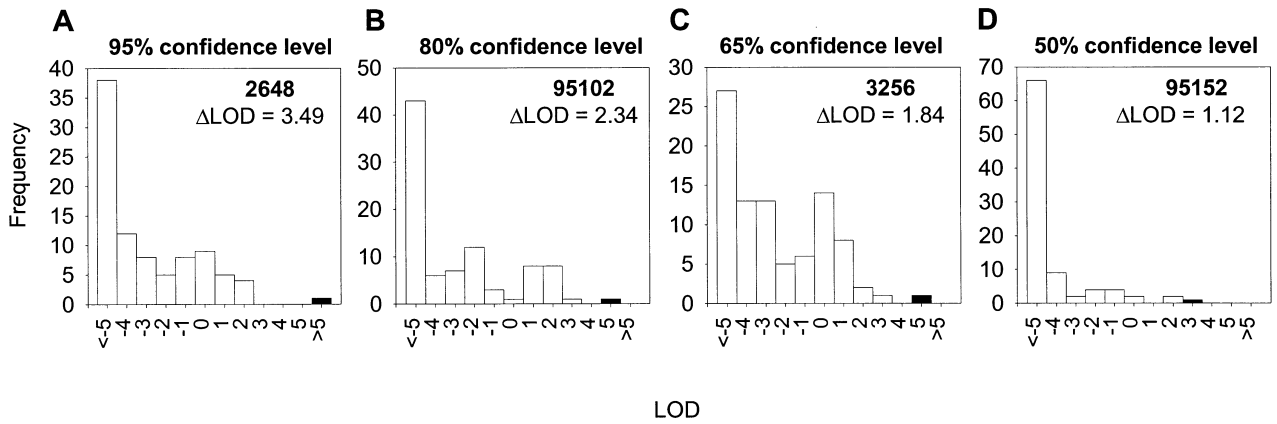

Fig. 2 Examples of distributions of LOD scores for all sampled candidate males ($n = 90$) for pups with paternity assigned at (a) 95% confidence, (b) 80% confidence, (c) 65% confidence, and (d) 50% confidence. The LOD score of the most-likely candidate male is shown in black.

homozygous loci (U_{90} = 629, 619, and 587 for number of paternities assigned at 50, 65 and 80 confidence, $P > 0.05$ for each test).

Distribution and variance in male mating success

The distributions of paternities among males assigned at 50, 65 and 80% confidence are shown in Fig. 3. Only one paternity could be assigned at the 95% confidence level in either year. At all levels of statistical confidence, most males were assigned the paternity of zero or one pup, implying a low variance in male mating success. The mean and variance in male mating success among sampled males in each year and combined over both years are shown in Table 3. Also shown is the standardized variance in mating success, which is a measure previously used to compare levels of polygyny inferred from copulations between studies (Boness *et al*. 1993). A truly monogamous mating system will have a variance of zero, whereas mating systems with moderate to strong levels of polygyny have standardized variances ranging from five to 50 (Boness *et al*. 1993).

The simulation analyses indicated that the genetic system was incapable of discriminating between monogamy and a slight level of polygyny (mean of two offspring per successful male) at any confidence level (Fig. 4). The mean standardized variance in the number of paternities per male at greater levels of simulated reproductive skew was two- to fourfold greater than that in the monogamous simulations, indicating that moderate levels of polygyny would be detectable. The observed values at 50, 65 and 80% in both years fall in the same range as the simulations representing monogamy (one offspring per male) and slight polygyny (mean of two offspring per successful male; Fig. 4).

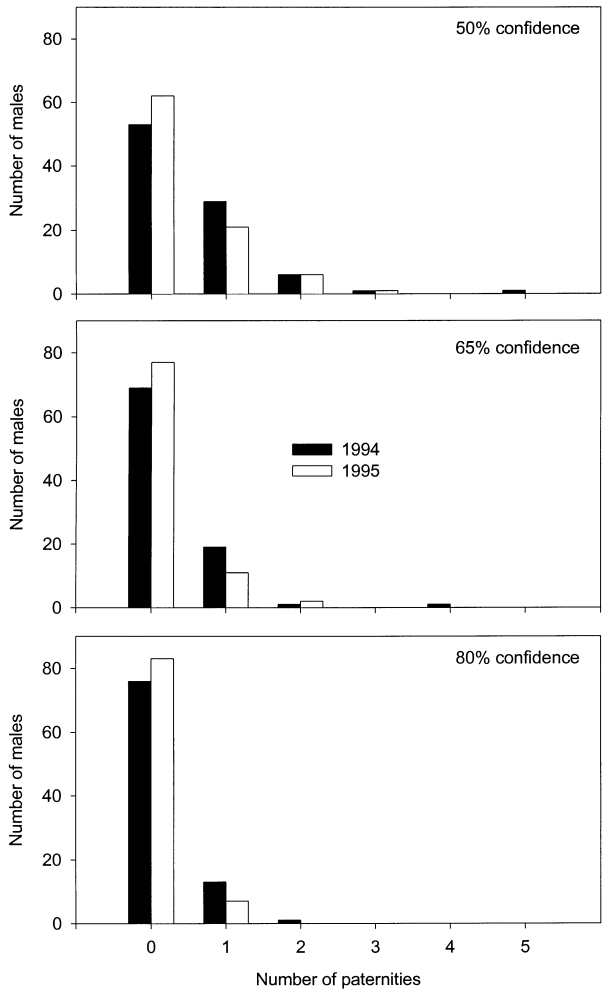

Fig. 3 Distribution of paternities among 90 male harbour seals assigned at (a) 50% confidence, (b) 65% confidence, and (c) 80% confidence in the 1994 and 1995 pup cohorts born at Sable Island, Nova Scotia.

Table 3 Summary statistics for estimates of male mating success under varying paternity assignment confidence criteria in the 1994 and 1995 cohorts. The standardized variance is the variance divided by the mean. Statistics are also shown for paternity data combined between both cohorts

Cohort year and confidence level	No. of paternities assigned	Maximum per male	Mean per male	Variance	Standardized variance
1994 (n = 144)					
50%	49 (34.0%)	5	0.54	0.68	1.26
65%	25 (17.4%)	4	0.28	0.36	1.29
80%	15 (10.4%)	2	0.17	0.16	0.94
1995 (n = 131)					
50%	36 (27.5%)	3	0.40	0.44	1.10
65%	15 (11.4%)	2	0.17	0.19	1.12
80%	7 (5.3%)	1	0.08	0.07	0.88
Combined (n = 275)					
50%	85 (30.9%)	6	0.94	1.08	1.15
65%	40 (14.5%)	4	0.42	0.52	1.24
80%	22 (8.0%)	2	0.24	0.23	0.96

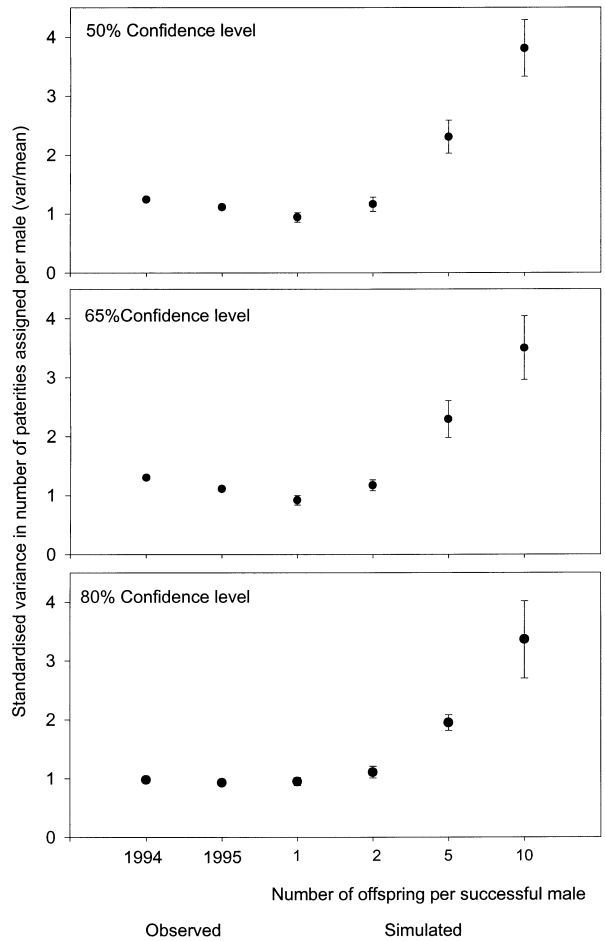

Fig. 4 Standardized variances in the number of paternities assigned to males at 50, 65 and 80% confidence levels. Data shown are observed values for the 1994 and 1995 cohorts, and values from simulations with varying levels of reproductive skew (mean of 1, 2, 5 and 10 offspring sired by each successful male).

Estimation of paternal half sibs within cohorts and between seasons

Estimates of G, G_P and the observed frequencies of shared paternally derived alleles among pups typed at all six loci are shown in Table 4. At each locus, the level of paternally derived allele sharing between pups within cohorts tended to be lower than that expected for paternally unrelated sibs. However, only the difference observed at SGPV 11 was statistically significant ($t_{9180} = 8.08$ and $t_{7513} = 3.75$, $P < 0.001$ for each year). At β-globin, *Pvc* 43 and SGPV 3 the frequency of shared paternally derived alleles between maternal sibs was slightly higher than G, and slightly lower at H.g. 8.10, *Pvc* 19 and SGPV 11; however, these differences were not statistically significant.

The frequency distributions of observed levels of paternally derived shared alleles between pups in the 1994 and 1995 cohorts, and between maternal half sibs, are illustrated in Fig. 5. Also shown are the distributions of expected levels of paternal allele sharing for paternally unrelated and paternal half sibs. These were generated assuming that levels of allele sharing are distributed normally, with a mean and standard deviation corresponding to G and G_P, respectively (Table 4). The observed distributions of paternal allele sharing completely overlap the range of allele sharing expected for paternally unrelated individuals. The best estimate for the frequency of paternal half sibs is approximately zero both within cohorts and among maternal half sibs, combining the probability values for all loci (Fig. 6). The upper 95% confidence limit, taken as three log units above the maximum likelihood solution, for the 1994 and 1995 cohorts is 10 and 12%, respectively, and 29% for maternal half sibs.

Locus	G	G_p	Frequency of paternally derived sharded alleles		
			1994 cohort (N = 9180)	1995 cohort (N = 7513)	Maternal sibs (N = 59)
β-globin	0.286	0.643	0.258	0.247	0.355
	(0.037)	(0.039)	(0.008)	(0.006)	(0.112)
H.g. 8.10	0.476	0.738	0.483	0.467	0.381
	(0.040)	(0.037)	(0.007)	(0.008)	(0.095)
Pvc 19	0.572	0.786	0.548	0.522	0.492
	(0.039)	(0.039)	(0.008)	(0.008)	(0.102)
Pvc 43	0.455	0.728	0.418	0.401	0.492
	(0.042)	(0.037)	(0.008)	(0.008)	(0.108)
SGPV 3	0.119	0.560	0.110	0.103	0.169
	(0.014)	(0.021)	(0.006)	(0.006)	(0.090)
SGPV 11	0.595	0.798	0.426	0.517	0.453
	(0.040)	(0.033)	(0.009)	(0.008)	(0.114)
Combined	0.417	0.709	0.374	0.376	0.395
	(0.145)	(0.074)	(0.004)	(0.003)	(0.037)

Table 4 Population estimates of gene identity for unrelated (G) and paternal half sibs (G_p) with the observed frequencies of shared paternally derived alleles between pups among cohorts and born to the same female in consecutive years. 95% confidence limits are shown in parentheses

Discussion

The limitations of paternity analysis

The results of this study suggest that six microsatellite loci with a combined probability of exclusion of ≈ 95% are inadequate for defining pedigree relationships at a level of 95% confidence in a natural population where there are a large number of candidate males, many of which are unsampled. More paternities could be assigned by improving the resolution of the genetic system (i.e. adding more polymorphic loci), or by sampling more candidate males.

Over the course of this study, primers for 32 pinniped microsatellite loci were screened for polymorphism in this population (Sable Island harbour seals: n = 12, Coltman *et al*. 1996; D. W. Coltman unpublished data; other species including harbour seals: n = 20, Gemmell *et al*. 1997 and references therein). We used loci with at least two alleles and observed heterozygosities of greater than 0.45. Most loci were mono- or dimorphic, with lower levels of heterozygosity in the Sable Island population than in other species (Coltman *et al*. 1996), suggesting that this population has relatively low genetic variability which makes precise paternity assignment difficult given the large number of possible sires. The large number of males which were assigned no paternities at all is thus partially a consequence of the limited number of paternities which could be assigned. The likelihood approach should be more resolving in other datasets which have greater power than this one, or which have fewer candidate sires to discriminate.

Sampling a greater number of candidate males would improve the success rate to a certain extent. However, the degree of disturbance this would necessitate to make a substantial improvement in our representation of the adult male population would be unacceptable during the breeding season. Noninvasive sampling methods, such as typing males from faeces (e.g. Reed *et al*. 1997), provide an alternative method to increase the male sample size; however, the identity of the sampled individuals would be unknown.

Our approach to the limitations of the genetic data was to assign paternities at less than 95% confidence. We feel this is justified for several reasons. First, other published paternity studies have either not estimated the statistical level of confidence in assigning paternity using simple most-likely male criteria (e.g. Meagher 1986), have tested the significance of LOD scores of most-likely males by conversion to χ^2 followed by Bonferroni correction for multiple comparisons (e.g. Taylor *et al*. 1997), or have simply assigned paternities to unique nonexcluded males (Morin *et al*. 1994; Craighead *et al*. 1995). None of these approaches consider the problems of unsampled males and false paternity exclusions due to typing errors which are simulated in the CERVUS program (Marshall *et al*. 1998).

Second, for a species in which it is impossible to observe copulations, a measure of mating success with a predicted error rate 50% or less provides useful biological information. If false positives are distributed randomly among males, inferences made from the genetic data, such as associations between phenotype and mating success, will be conservative due to random noise in the genetic data. For studies of heritability, which may require precise pedigree knowledge, it may be necessary to use more stringent paternity assignment criteria than for descriptive studies of mating systems in natural populations.

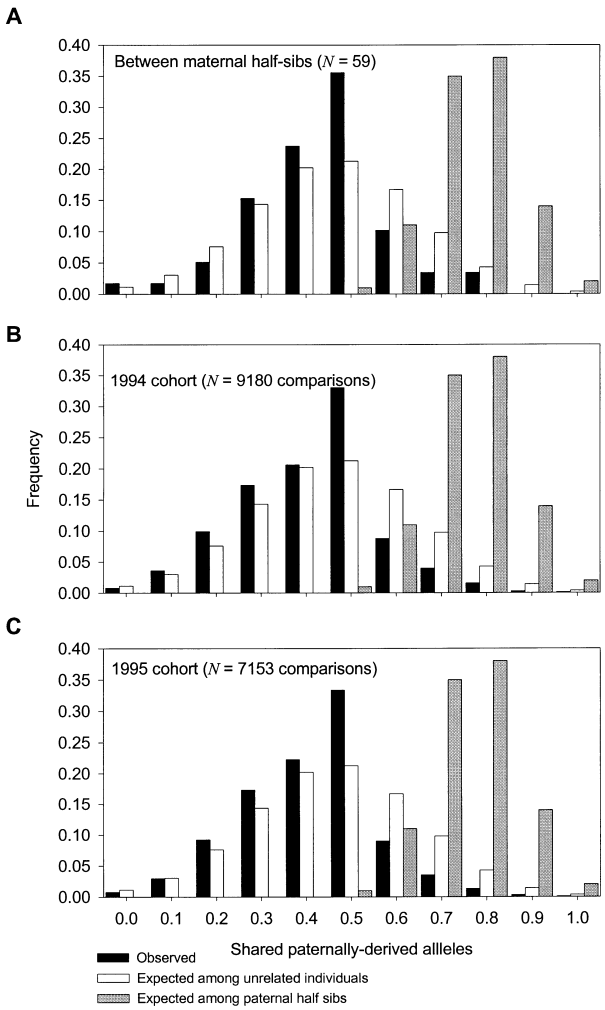

Fig. 5 Distribution of the average frequency of shared paternally derived alleles at six microsatellite loci between (a) 59 maternal half sibs born in 1994 and 1995, and among all pups born in (b) 1994 (all pairwise comparisons of 136 pups), and in (c) 1995 (all pairwise comparisons of 123 pups). Also shown are the distributions of levels of paternally derived allele sharing expected for paternally unrelated and paternal half sibs.

The variance in mating success among male harbour seals

Regardless of the paternity assignment criteria, the estimated variance in mating success among the sampled male harbour seals was low (Table 4) with the majority of the successful males expected to have sired a single pup (Fig. 3). The simulations indicated that it is unlikely that a considerable degree of reproductive skew was undetected by the paternity analysis among the sampled males. Also, the estimated proportion of paternal half sibs within cohorts was not significantly different from zero in either cohort (Fig. 6), suggesting that it was unlikely that a small number of unsampled males were highly successful. At Sable Island, harbour seals may be considered polygynous in that some males are likely to sire more than one pup within a breeding season (Fig. 3). However, the level of polygyny must be considered slight, approaching that of genetic monogamy (Fig. 4). Compared to data based on observed copulations in other pinniped species, the harbour seal thus falls at the lower end of a continuum of polygyny (Boness *et al*. 1993). Data based on behavioural observation should, however, be viewed with caution. For example, a moderate variance in male mating success (3.0) based on observed copulations was found by Anderson *et al*. (1975) among male greys seals at North Rona. Genetic data from Amos *et al*. (1993) indicated a much lower variance (0.5) than suggested by Anderson *et al*. (1975), as Amos *et al*. (1993) found that the mating success of the dominant males was not as great as the number of copulations witnessed would suggest.

The variance in male mating success in aquatically mating species is generally lower than it is in species

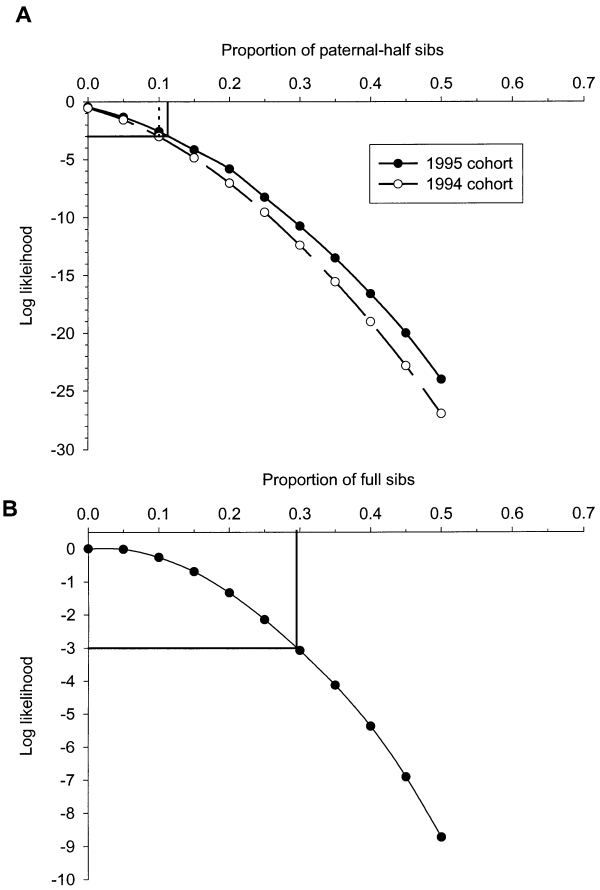

Fig. 6 Maximum likelihood estimate of the proportion of (a) paternal half sibs occurring within the 1994 and 1995 cohorts, and (b) full sibs born to the same female in 1994 and 1995 at Sable Island, Nova Scotia. The upper 95% confidence limit, estimated as 3 log units above the mean, is shown for each curve.

which mate on land. However, there is a wide range in the degree of polygyny evident among terrestrially mating pinnipeds (Boness 1991; Boness *et al*. 1993). The physical structure of the mating environment (i.e. the added spatial dimension of aquatic over terrestrial habitats) clearly influences the distribution and defencibility of receptive females in time and space, yet among populations there are other ecological factors which determine the degree to which males can monopolise access to receptive females (Le Boeuf 1991; Boness 1991). In the case of Weddell seals and aquatically mating Juan Fernandez fur seals, there are relatively well-defined, defensible local resources (leads in the ice and sheltered thermoregulatory sites near the breeding colony, respectively) which attract potentially receptive females during the breeding season (Hill 1987 and Francis & Boness 1991; Bartsh *et al*. 1992). Males which successfully defend these sites either may have achieved more copulations than nonterritorial males as inferred from coloured grease transfer experiments in Weddell seals (Hill 1987; Bartsh *et al*. 1992), or attended as many females as did males which held terrestrial territories in the breeding colony (Juan Fernandez fur seals; Francis & Boness 1991). However, these examples may not be typical of other aquatically mating pinnipeds. Similar to harbour seals on Sable Island, female harp, *Phoca groenlandica*, hooded, *Cystophora cristata*, (Boness *et al*. 1988; Kovacs 1990) and crabeater seals, *Lobodon carcinophagus*, (Siniff *et al*. 1979) may be dispersed spatially during the breeding season. The variance in male mating success in these species may also be low.

In this study, the number of paternities males were assigned in one year was not correlated with mating success in the following year. Interannual variation in mating success may therefore have a homogenising influence on the degree of polygyny implied by estimates of the variance in mating success, when based on a small number of seasons. The lifetime reproductive output among individual males may thus vary less than single measurements of seasonal output would imply. Unfortunately, such long-term data is difficult to collect from organisms with reproductive lifespans which exceed the lifetime of most research projects. For pinnipeds, published lifetime reproductive success data are limited to a single species, the northern elephant seal (Le Boeuf & Reiter 1988). The vast majority of the estimates of polygyny and the descriptions of mating systems of pinnipeds and other species are based on observations of a single season (references in Boness *et al*. 1993) and it is becoming increasingly clear that lifetime data on reproductive success are necessary to gain a better understanding of selection and adaptation (Clutton-Brock 1988; Murray 1992). Seasonal data are valuable to the study of mating systems, however, as they describe the processes which act within a single episode of selection (i.e. each breeding season), and seasonal data are also useful for identifying traits which may ultimately be subject to sexual selection pressure.

Acknowledgements

We thank Daryl Boness, Sara Iverson, Jim McMillan and Monica Muelbert for assisting with sample collection at Sable Island. Logistic support was provided during fieldwork on Sable Island by Gerry Forbes, AES Canada. Doug Cook provided valuable PCR advice to DWC. Tristan Marshall, University of Edinburgh, provided useful advice regarding paternity analysis and the program CERVUS can be obtained from him (tristan.marshall@ed.ac.uk). We thank Drs Marty Leonard, Josephine Pemberton and two anonymous reviewers for useful comments on the manuscript. D.W.C. was supported by an NSERC postgraduate scholarship and this research was funded by NSERC operating grants to W.D.B. and J.M.W. and by the Department of Fisheries and Oceans, Canada.

References

Allen PJ, Amos W, Pomeroy PP, Twiss SD (1996) Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology*, **4**, 653–662.

Amos W, Twiss S, Pomeroy P, Anderson S (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 grey seal. *Science*, **268**, 1897–1899.

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

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

Bartholomew GA, Hoel PG (1953) Reproductive behaviour of the Alaska fur seal, *Callorhinus ursinus. Journal of Mammalogy*, **34**, 417–436.

Bartsh SS, Johnston SD, Siniff DB (1992) Territorial behaviour and breeding frequency of male Weddell seals (*Leptonychotes weddelli*) in relation to age, size, and concentrations of serum testosterone and cortisol. *Canadian Journal of Zoology*, **70**, 680–692.

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

Boness DJ, Bowen WD, Oftedal OT (1988) Evidence of polygyny from spatial patterns of hooded seals (*Cystophora cristata*). *Canadian Journal of Zoology*, **66**, 703–706.

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

Boulva J, McLaren IA (1979) Biology of the harbour seal (*Phoca vitulina*), in eastern Canada. *Bulletin of the Fisheries Research Board of Canada*, **200**, 1–24.

Clutton-Brock TH (1988) Reproductive success. In: *Reproductive Success* (ed. Clutton-Brock TH), pp. 472–487. University of Chicago Press, Chicago.

Coltman DW, Bowen WD, Boness DJ, Iverson SJ (1997) Balancing foraging and reproduction in male harbour seals: an aquatically mating pinniped. *Animal Behaviour*, **54**, 663–678.

Coltman DW, Bowen WD, Iverson SJ, Boness DJ (1998) The energetics of male reproduction in an aquatically mating pinniped: the harbour seal. *Physiological Zoology*, **71**, in press.

Coltman DW, Bowen WD, Wright JM (1996) PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in several pinniped species. *Molecular Ecology*, **5**, 161–163.

Craighead L, Paetkau D, Reynolds HV, Vyse ER, Strobeck C (1995) Microsatellite analysis of paternity and reproduction in Arctic grizzly bears. *Journal of Heredity*, **86**, 255–261.

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

Francis J, Boness DJ (1991) The effect of thermoregulatory behaviour on the mating system of the Juan Fernandez fur seal, *Arctocephalus phillippii*. *Behaviour*, **199**, 104–127.

Gemmell NJ, Allen PJ, Goodman SJ, Reed JZ (1997) Interspecific microsatellite markers for the study of pinniped populations. *Molecular Ecology*, **6**, 661–666.

Godsell J (1988) Herd formation and haul-out behaviour in harbour seals (*Phoca vitulina*). *Journal of Zoology London*, **215**, 83–98.

Goodman SJ (1997) Dinucleotide repeat polymorphism at 7 anonymous microsatellite loci cloned from the European harbour seal (*Phoca vitulina vitulina*). *Animal Genetics*, **28**, 310–311.

Hill SEB (1987) Reproductive ecology of Weddell seals (*Leptonychotes weddelli*) in McMurdo Sound, Antarctica. PhD thesis, University of Minnesota, Minnesota, USA.

Kovacs KM (1990) Mating strategies in male hooded seals *(Cystophora cristata*)? *Canadian Journal of Zoology*, **68**, 2499–2502.

Le Boeuf BJ (1974) Male-male competition and reproductive success in elephant seals. *American Zoologist*, **14**, 163–176.

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

Le Boeuf BJ, Reiter J (1988) Lifetime reproductive success in male and female northern elephant seals. In: *Reproductive Success* (ed. Clutton-Brock TH), pp. 344–362. University of Chicago Press, Chicago.

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

McLaren IA (1993) Growth in pinnipeds. *Biological Reviews*, **68**, 1–79.

Meagher TR (1986) Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. *American Naturalist*, **128**, 199–215.

Morin PA, Wallis J, Moore JJ, Woodruff DS (1994) Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats. *Molecular Ecology*, **3**, 469–478.

Murray Jr BG (1992) The evolutionary significance of lifetime reproductive success. *Auk*, **109**, 167–172.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **83**, 239.

Reed JZ, Tollit DJ, Thompson PM, Amos W (1997) Molecular scatology: the use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. *Molecular Ecology*, **6**, 225–234.

Siniff DB, Stirling I, Bengtson JL, Reichle RA (1979) Social and reproductive behaviour of crabeater seals (*Lobodon carcinophagus*) during the austral spring. *Canadian Journal of Zoology*, **57**, 2243–2255.

Sokol RR, Rohlf JF (1995) *Biometry*, 3rd edn. Freeman, New York.

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

Taylor AC, Horsup A, Johnson CN, Sunnucks P, Sherwin B (1997) Relatedness structure detected by microsatellite analysis and attempted pedigree reconstruction in an endangered marsupial, the northern hairy-nosed wombat *Lasiorhinus krefftii*. *Molecular Ecology*, **6**, 9–19.

Thompson EA (1976) Inference of genealogical structure. *Social Science Information*, **15**, 477–526.

Thompson PM (1988) Timing of mating in the common seal (*Phoca vitulina*). *Mammalian Reviews*, **18**, 105–112.

Trivers RL (1972) Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man, 1871–1971* (ed. Campbell B), pp. 136–179. Aldine, Chicago.

Walker BG, Bowen WD (1993) Behavioural differences among adult male harbour seals during the breeding season may provide evidence of reproductive strategies. *Canadian Journal of Zoology*, **71**, 1585–1591.

Weir B (1990) *Genetic Data Analysis*. Sinauer, Massachusetts.

This study was part of D. W. Coltman's PhD thesis research conducted at Dalhousie University. D. W. Coltman is now a postdoctoral fellow at Edinburgh University studying the molecular ecology of Soay sheep, but maintains an active interest in pinniped biology. W. D. Bowen is a research scientist at the Department of Fisheries and Oceans, Canada, specializing in the ecology and life history of marine mammals. J. M. Wright is a professor of biology at Dalhousie University with a wide range of genetic research interests.
