

# The rate of fertilization in male mating tactics of the polygynous grey seal

D. C. LIDGARD,\*†‡ D. J. BONESS,† W. D. BOWEN,‡ J. I. McMILLAN‡ and R. C. FLEISCHER§

\*Laboratoire de Biologie et Environnement Marins, Université de La Rochelle, La Rochelle F-17000, France; †Conservation and Research Center, National Zoological Park, Smithsonian Institution, Washington, DC 20008-2598, USA; ‡Marine Fish Division, Bedford Institute of Oceanography, Department of Fisheries and Oceans, Dartmouth, Nova Scotia, Canada B2Y 4A2; §Genetics Program, Department of Systematic Biology, National Museum of Natural History, Smithsonian Institution, 3001 Connecticut Avenue, NW, Washington, DC 20008-0551, USA

## Abstract

**Studies using molecular markers have shown that some grey seal males may be gaining success through exhibiting alternative mating tactics. We estimated the probability of fertilization success of grey seal males exhibiting the primary tactic of female defence and one alternative tactic of mating with departing females on Sable Island, Nova Scotia, Canada, during the breeding seasons of 1997–2002. Although the fertilization rate of the primary tactic (27–43%) was greater than that of the alternative tactic (10–12%), these low rates indicate the potential fitness value of alternative mating tactics in this size-dimorphic pinniped species.**

*Keywords:* alternative mating tactics, microsatellites, paternity, pinnipeds, reproductive behaviour

*Received 25 May 2004; revision received 9 July 2004; accepted 26 July 2004*

## Introduction

In mating systems characterized by weak dominance relationships among males, brief male–female associations or strong female promiscuity, males may increase their fitness through exhibiting alternative mating tactics (Coltman *et al.* 1999). The grey seal (*Halichoerus grypus*) is a sexually dimorphic pinniped with a polygynous mating system (Anderson *et al.* 1975; Boness & James 1979). Males arrive at the breeding colony with large quantities of stored energy. The primary mating tactic is prolonged female defence whereby a male defends a position among a group of females (Anderson *et al.* 1975; Boness & James 1979). Males also exhibit alternative mating tactics which are not confined to young and small males (Lidgard 2003). One such tactic involves males mating with females that have weaned their offspring and are leaving the colony. Le Boeuf & Mesnick (1990) and Wainstein (2000) have observed this tactic in northern and southern elephant seals (*Mirounga* spp.), respectively. The occurrence of this tactic in other

species of pinniped is unknown since the majority of studies on the reproductive behaviour of male pinnipeds have focused on the primary tactic. Several genetic studies have indirectly suggested that alternative tactics in the grey seal and other pinnipeds are successful (Amos *et al.* 1993; McRae & Kovacs 1994; Coltman *et al.* 1998; Ambs *et al.* 1999; Hoelzel *et al.* 1999; Worthington Wilmer *et al.* 1999) and may therefore provide a significant contribution to the gene pool. However, no studies have directly measured their success. Indeed, within mammals the success of alternative mating tactics is poorly understood.

The aim of this study is to estimate the success of the primary male mating tactic and of the alternative tactic of mating with departing females in the grey seal on Sable Island. Our initial intention was to estimate the success of each tactic for a sample of focal males as part of a larger study investigating the importance of male phenotype and behaviour on reproductive success. However, owing to the difficulty of capturing departing females and relocating females in the following season to obtain a skin sample from the pup, we were unable to sample all potential mates for each male. Rather, we grouped paternity data from a sample of focal males and estimated the percentage of offspring sired to provide an estimate of the probability of fertilization success for each tactic.

Correspondence: D. C. Lidgard. ‡Present address: Sea Mammal Research Unit, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland, UK. Fax: +44 (0)1334 462632; E-mail: dcl2@st-andrews.ac.uk

## Materials and methods

The study was conducted during the breeding seasons of 1997–2002 on Sable Island, Nova Scotia, Canada (43°55' N, 60°00' W) between late December and early February. The number of pups born in this population has increased at an annual rate of 13% for the past four decades (25 400 pups in 1997; Bowen *et al.* 2003). Males that were uniquely hot-iron branded at weaning since the late 1960s provided a pool of known-age adults.

Two sampling approaches were used to obtain estimates of fertilization success.

### Focal males

The first approach (Table 1) was cross-sectional in design and based on daily observations of 42 branded males for the entire breeding season (*see* Lidgard *et al.* 2001 for methodology). Focal males were located between one and three times daily, during which time the likelihood of the focal male mating with lactating or departing females was assessed based on the number of sightings he had spent with the female and observed attempts at copulation (Lidgard *et al.* 2001). Females were categorized as either consorts of the male (nearest neighbour male to female for  $\geq 2$  d) or as departing (*see definition below*). Females that were considered to be potential mates were captured and a skin sample was taken from the hind-flipper. To collect a skin sample from the female's pup in the following breeding season, either a supertag (Dalton Supplies Ltd, Nettlebed, Henley-on-Thames, UK) was attached to the webbing of the female's hind flipper or an ankle tag was secured. To attach ankle tags, females were sedated with

5 mL of diazepam (5 mg/mL). Flipper tags and anklets were removed when the female was relocated with her pup in the subsequent breeding season.

Genotypic data from cases where focal males mated with departing females were grouped with data from the study that was specifically designed to estimate the success of this tactic (*see below*). Given the large size of the Sable Island population, we were unable to relocate a number of the females. To overcome the small sample size resulting from this difficulty, we combined genotypic data from those focal males that mated with their consorts (i.e. consort males) with genotypic data from a previous study by our research group on Sable Island that was designed to estimate the frequency of extra-consort fertilizations (ECFs) in grey seals (Ambs *et al.* 1999). Although Ambs *et al.* (1999) focused on females and sampled each consort male, by randomly choosing one of the males as the possible father, paternity could be assigned using the same method as in the present study (*see below*).

Combining data from the two studies seems reasonable because females from our study should be just as likely to mate with other consort or nonconsort males as females in Ambs *et al.* (1999). In addition, there is no a priori reason to expect differences in the duration of consortship or the range of locations used by individuals in both studies. The latter point is important because Ambs *et al.* (1999) found that location can affect the likelihood of extra-consort fertilizations.

### Departing females

This approach (Table 1) was designed to estimate the probability of fertilization success for males mating with

**Table 1** Years of study, sampling methods and sample sizes for the three datasets used in this study to estimate probabilities of fertilization success for the primary mating tactic of consortship and the alternative tactic of mating with females departing the colony for grey seal males on Sable Island

Mating tactic	Mating with consort females		Mating with departing females	
	Focal male study (this study)	Focal female study (Ambs <i>et al.</i> 1999)	Departing female study (this study)	Focal male study (this study)
Study years (excluding relocating females in the following season)	1997–2001	1995	2000	1997–2001
Reason for sampling a male	Nearest neighbour male to female for $\geq 2$ d	Nearest neighbour male to female for $\geq 2$ d	Observed with departing female	Observed with departing female
Number of males sampled per female	1	1–3, median 2	1	1
Method of relocating females	Flipper and ankle tags	Brands and radio tags	Brands	Flipper and ankle tags
Number of males sampled	42 (14)	77 (77)	76 (43)	42 (13)
Number of females relocated and sampled	23 (22)	24 (15)	52 (43)	18 (17)
Number of pups sampled	25 (22)	24 (15)	52 (43)	20 (17)

Numbers in brackets are number of typed individuals used in analyses.

**Table 2** The number of alleles, annealing temperature, per cent observed and per cent expected heterozygosity, Hardy–Weinberg equilibrium probabilities, individual (*PI*) and combined (*P*) probability of exclusion for seven microsatellite loci from 166 individuals

Locus	Number of alleles	Annealing temperature (°C)	% Observed heterozygosity ( $H_O$ )	% Expected heterozygosity ( $H_E$ )	Hardy–Weinberg equilibrium probabilities	Probability of exclusion ( <i>PI</i> )
Hg6.3	7	58	83.1	73.5	0.27	0.509
Hg8.9	8	54	71.1	72.9	0.64	0.519
Hg6.1	5	60	65.7	66.9	0.27	0.385
Hgdii	10	50	77.7	75.9	0.53	0.552
Hg3.6	6	62	83.1	77.1	0.33	0.549
Nes-M11a	3	50	48.2	49.4	0.95	0.194
Pvc63	5	45	35.5	63.9	< 0.0001	—
<i>P</i>						0.976

Per cent heterozygosity and Hardy–Weinberg equilibrium probabilities were calculated using GENEPOP 3.1 (Raymond & Roussett 1995).

females that had weaned their pup and were departing the colony. In this tactic, males are promiscuous and do not defend females but actively pursue them as they leave the colony and compete for access to the female among many other males. The subset of individuals used in this analysis was separate from that used in the focal male study. Using a pole with a biopsy punch fastened to the end, skin samples were taken from males observed with departing branded females. Because females on Sable Island remain with their pup throughout the lactation period (Boness & James 1979), females were deemed to be departing after successful lactation if they were thin, not with their pup and moving toward the sea. Only uniquely branded females were sampled to increase the chances of locating them in the following season.

#### DNA extraction and microsatellite typing

Samples were preserved in vials containing 20% dimethylsulphoxide (DMSO) in a salt-saturated solution and stored at  $-20^{\circ}\text{C}$  (Amos & Hoelzel 1991). Total genomic DNA was extracted using a commercial DNA kit (DNeasy Tissue Kit; Qiagen, Inc., Valencia, CA, USA). Isolated DNA was genotyped with seven polymorphic dinucleotide repeat microsatellite loci. These loci were isolated from the eastern Atlantic grey seal (Hg3.6, Hgdii, Hg6.1, Hg6.3, Hg8.9; Allen *et al.* 1995), harbour seal (*Phoca vitulina*) (Pvc63; Coltman *et al.* 1996) and the northern elephant seal (*Mirounga angustirostris*) (NES-M11A; Gemmell *et al.* 1997) (Table 2). Polymerase chain reaction (PCR) amplifications were performed in 20- $\mu\text{L}$  reaction volumes, each containing 1  $\mu\text{L}$  of forward and reverse primer (10 mM), 1  $\mu\text{L}$  of buffer, 1  $\mu\text{L}$  of a 2 mM mix of dGTP, dATP, dTTP and dCTP, 0.8–1.6  $\mu\text{L}$  of 25 mM  $\text{MgCl}_2$ , 0.1–0.2  $\mu\text{L}$  *Taq* polymerase, 1–2  $\mu\text{L}$  of DNA (50–100 ng) and sterile double-distilled water to 20  $\mu\text{L}$ . Thermal cycling was as follows: 2.5 mins at  $93^{\circ}\text{C}$  followed by 32 cycles of 45 s of denaturing at  $93^{\circ}\text{C}$ , 45 s at the specified annealing temperature (Table 2) and 45 s of extension at  $72^{\circ}\text{C}$ . Microsatellites were visualized either

on a 5% polyacrylamide gel on an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the marker GS500 TAMRA, or on an ABI PRISM 3100 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the marker GS500 ROX. In both cases, at least one size standard of known genotype was run for each primer and gel. Loci were scored using GENE SCAN ANALYSIS version 3.7 and GENOTYPER version 1.1 software (Applied Biosystems, Foster City, CA, USA).

#### Paternity analysis

We used genotypic data from all typed individuals, excluding pups ( $n = 166$ ), to calculate Hardy–Weinberg equilibrium probabilities, linkage disequilibrium, and allele frequencies for each locus using GENEPOP version 3.1 (Raymond & Roussett 1995; Table 2). Pvc63 deviated significantly from Hardy–Weinberg equilibrium, which was likely due to the presence of null alleles, and was excluded from the analysis. Males used in Ambs *et al.* (1999) were scored with seven loci (see paper for details).

A male was excluded as the father if he did not have the paternal allele at any one of the six loci. Knowing the identity of the mother, we calculated the average probability of excluding an unrelated male as the father at each loci, *l*, according to:

$$PI = a_1 - 2a_2 + a_3 + 3(a_2a_3 - a_5) - 2(a_2^2 - a_4)$$

where  $a_n = \sum_{i=1}^k p_i^n$  and  $p_i$  is the frequency of allele *i*, and  $a_1 = 1$  (Chakravarti & Li 1983). We then calculated the overall average probability of exclusion, *P*, for the six loci according to:

$$P = 1 - \prod_{i=1}^n [1 - PI]$$

Paternity was also assigned using CERVUS 1.0 (Marshall *et al.* 1998). This program assigns paternity using a likelihood

approach whereby a simulation calculates critical log-likelihood scores ( $\Delta$ LOD) for each candidate male and assigns paternity at a given level of statistical confidence. To determine if the study male was the most likely father we conducted a five-candidate male simulation for each male–female–pup triad for this study and for the study of Ambs *et al.* (1999). Female grey seals mate with a mean of 1.7 different males (range 1–5) before leaving the breeding colony (Ambs *et al.* 1999). To provide a conservative measure we assumed each female had mated with five different males. Critical  $\Delta$ LOD scores were generated from 10 000 paternity simulations where we assumed that 99% of loci were typed and a 1% error rate. Given that only one male was sampled for each female, the proportion of candidate males sampled was 0.2. The number of study males assigned paternity was given at 95% and 80% confidence.

## Results

### Focal males

During the study period, 42 males were sampled. A total of 184 females were marked and identified as possible mates and of these, 41 were relocated in the following season and a skin sample taken from their pup. In four cases, a female was observed with two pups at the time of sampling. In two of these cases, the genetic pup was identified using the six loci. In the other two cases, it was not possible to reject either of the two pups and these two triads were excluded from the analysis. On Sable Island, females have been observed with two young pups raising the possibility that females may give birth to twins (Jim McMillan, pers. comm.).

Fourteen males and 22 mother–pup pairs comprised the sample of consort matings; five males were associated with two or more mother–pup pairs. We assumed that the number of different females a consort male copulated with did not influence the likelihood of paternity. These data were combined with the genotypic data of 15 male–female–pup triads obtained from Ambs *et al.* (1999). From matching

the pup's genotype with that of the putative father's genotype, in 12 of 37 cases the male was not excluded as the father. The number of focal males assigned a paternity by CERVUS was 10 (27%) at 95% confidence and 16 (43%) at 80% confidence (Table 3). The number of males that were excluded because of a single mismatch but assigned a paternity by CERVUS was one at 95% and two at 80% confidence.

### Departing females

Seventy-six males were observed with departing females, and 52 mother–pup pairs were relocated and sampled in the following season. The genotype of three mother–pup pairs did not match for either allele at all loci and were excluded from the analysis. It was assumed that the female was nursing a nonfilial pup, which is known to occur on Sable Island (Perry *et al.* 1998). Six males and their associated mother–pup pairs were excluded from the analysis because the genotype of one male could not be identified because two primers failed to amplify, and in the other five cases only hair was sampled, thus no DNA could be extracted (Table 1).

Thirteen males and 17 mother–pup pairs from the focal male study were the result of males mating with departing females; three males were associated with two or more mother–pup pairs. Genotypic data from these triads were grouped with the 43 male–female–pup triads from the departure female study to gain a better estimate of success for this tactic. From matching the pup's genotype with that of the alleged father's genotype, in five of 60 cases the male was not excluded as the father. The number of males assigned a paternity by CERVUS was six (10%) at 95% confidence and seven (11.7%) at 80% confidence (Table 3). The number of males that were excluded because of a single mismatch but assigned a paternity was one at 95% and two at 80%. At both levels of confidence, the fertilization success of this tactic was significantly lower than the success of the consort tactic (Table 3). The fertilization success of these two tactics also differed statistically when we compared the observed exclusion rates (32.4% vs. 8.3%;  $\chi^2_1 = 9.01$ ,  $P = 0.01$ ).

**Table 3** Summary of paternity analysis using CERVUS 1.0 (Marshall *et al.* 1998) for the primary mating tactic of consortship and the alternative tactic of mating with females departing the colony for grey seal males on Sable Island

Confidence level (%)	Mating with consort females				Mating with departing females					
	This study		Ambs <i>et al.</i> (1999)		Probability of fertilization	This study			$\chi^2_1$	$P$
	Number of males assigned paternity	$\Delta$	Number of males assigned paternity	$\Delta$		Number of males assigned paternity	$\Delta$	Probability of fertilization		
95	4 (22)	2.48	6 (15)	2.47	27.0%	6 (60)	2.48	10.0%	4.7	< 0.05
80	9 (22)	0.89	7 (15)	0.68	43.2%	7 (60)	0.89	11.7%	12.6	< 0.01

$\Delta$  represents the critical  $\Delta$ LOD score;  $\chi^2_1$  and  $P$ -values refer to a  $\chi^2$  analysis between the two tactics.

## Discussion

### *The limitations of paternity analysis*

A major difficulty with this study was obtaining a sufficient sample size for paternity analysis. This was largely due to the difficulty of relocating females in the following season to obtain a skin sample from the pup. A high rate of tag loss and the large size of the Sable Island grey seal colony were primarily responsible for the number of females that could be relocated. As explained earlier, we feel confident in combining our data with that from Ambs *et al.* (1999).

Using more than six molecular markers and sampling other candidate males in addition to the focal male for each female would have improved our confidence in assigning paternities. Initially, we used seven markers but Pvc63 deviated significantly from Hardy–Weinberg equilibrium and was excluded. Given the transient nature of males in the colony (Lidgard *et al.* 2001), the number of females in the study and our commitment to monitoring the daily movements of each focal male and their associations with females, it was logistically not possible to assess the number of candidate males and sample them. It is therefore possible, that an unsampled male closely related to the sampled male sired the offspring and paternity was awarded to the sampled male. However, given the size of the colony and the transient nature of males we consider this to be unlikely. Nevertheless, the error in assigning paternities is likely to be random across the two tactics and thus we feel we can still have confidence in the biological pattern.

Another difficulty was determining the number of males that a female may mate with to provide a rigorous analysis in CERVUS. We chose five candidate males since Ambs *et al.* (1999) found that female grey seals may mate with up to five males. If the analysis is repeated with 10 candidate males the level of success for each of the two tactics is not significantly different from the level of success obtained with five candidate males at either level of confidence (results not shown). Our assessments in the field of the likelihood of the focal male mating with lactating or departing females provide additional confidence in our paternity assignments.

### *The rate of fertilization success for the primary and alternative tactics*

We found that the primary tactic of mating with consort females yielded lower than expected rates of fertilization and hence lower estimates of reproductive success than based on behavioural studies (Anderson *et al.* 1975; Boness & James 1979). Similar results have been reported in grey seals from the UK (Amos *et al.* 1993; Worthington Wilmer *et al.* 1999) and in other species of pinnipeds (McRae &

Kovacs 1994; Coltman *et al.* 1998; Hoelzel *et al.* 1999). We have also shown that the tactic of mating with females that had weaned their pup and were departing the breeding colony yielded some success, although the fertilization rate was lower than that for the primary tactic.

The overall low rate of success of these two mating tactics suggest that two other alternative tactics on Sable Island (Lidgard 2003), for which we had inadequate samples to assess fertilization rates, likely yield some success. The first involves a consort male mating with neighbouring females (Boness & James 1979; Ambs *et al.* 1999). Ambs *et al.* (1999) suggests that neighbouring consort males may account for the majority of extra-consort fertilizations in grey seals on Sable Island. The second tactic involves a nonconsort male mating with a consort female on the periphery of a group or alone. Worthington Wilmer *et al.* (1999), in an attempt to explain the large proportion of unassigned paternities in grey seals, suggested that males might be mating with females at sea. This is another possible alternative tactic that might occur at Sable Island, but has yet to be investigated. Further work is needed to evaluate the potential success of these tactics. The low rates of fertilization observed in this study indicate the potential fitness value of alternative mating tactics in this size-dimorphic pinniped species.

## Acknowledgements

We are very grateful to Suzanne Ambs, Debbie Austin, Carrie Beck, Suzanne Budge, Dave Coltman, Steven Insley, Sara Iverson, Shelley Lang and Strahan Tucker for their assistance in the field. In particular, we express our thanks to Tom Hubbard and Tyler Schulz for their assistance in collecting skin samples. We are grateful for support provided on Sable Island by Gerry Forbes. Suzanne Budge and three anonymous reviewers provided valuable comments on the manuscript and improved its quality. We thank Tristan Marshall and Dave Coltman for providing advice on the use of CERVUS. The study was supported by a Smithsonian Institution Graduate and Pre-doctorate Fellowship awarded to DCL, and funds from the Friends of the National Zoo, the Smithsonian Institution, the Christensen Fund, the Canadian Department of Fisheries and Oceans and the National Science and Engineering Research Council of Canada.

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This study constitutes a chapter in the dissertation thesis of Damian Lidgard, who is interested in the reproductive behaviour of pinnipeds. Daryl Boness pioneered research on the breeding biology of the grey seal on Sable Island and continues to research the behavioural ecology of pinnipeds. Don Bowen is a population ecologist, interested in reproductive and foraging ecology, population dynamics and life history evolution of pinnipeds. Jim McMillan is a biologist at the Bedford Institute of Oceanography, Canada and has worked with the Sable Island grey seal program for 17 years. Rob Fleischer is an evolutionary and conservation biologist, and has long-term interests in the application of molecular markers to mating systems of pinnipeds and other vertebrates.

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