DNA fingerprinting evidence of nonfilial nursing in grey seals

ELIZABETH A. PERRY, DARYL J. BONESS and ROBERT C. FLEISCHER
Department of Zoological Research, National Zoological Park, Washington DC, 20008, USA

Abstract

We tested the hypothesis that kin selection may play a role in fostering behaviour in grey seals. Fostering frequency varied among three colonies, ranging from 3% to 28%. Band-sharing coefficients (S) of DNA fingerprints, from two multilocus probes, were used to predict relatedness (*r*). Mean *r* did not differ between foster mother-pup pairs and the expected *r* = 0 for presumed unrelated female-pup pairs. Likewise, mean *r* between fostered and filial pups compared to *r* between presumed unrelated pups within the same beaches did not differ. Mean S values of presumed unrelated pups on different beaches within the two smallest colonies were indistinguishable, indicating that there is not increased variation in relatedness in small colonies. These results suggest that kin selection does not play a significant role in the maintenance of grey seal fostering behaviour.

Keywords: DNA fingerprinting, fostering, grey seal, *Halichoerus grypus*, kin selection, nonfilial nursing

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Introduction

Fostering behaviour in mammals, i.e. care of young either in addition to or in place of that provided by the mothers, confers obvious advantages to offspring but would seem to be a costly behaviour for the caregivers. For mammalian females, the greatest energetic cost associated with reproduction is in milk production (Gittleman & Thompson 1988). Therefore, provisioning nonfilial young can result in increased costs through either a reduction in the transfer of nutrients to biological offspring or increased foraging efforts to replenish maternal stores. Because fostering is most common among social animals living in small groups, many authors have suggested that this behaviour may have evolved through kin selection or reciprocity (Riedman 1982). Others have suggested that fostering arises from maternal error or milk theft, especially in animals breeding in colonies (Boness *et al.* 1992; Packer *et al.* 1992).

Fostering occurs in many phocid seals (Riedman 1982; Stirling 1975) and is relatively common in some colonially breeding species (e.g. Riedman & LeBoeuf 1982; Kovacs

Correspondence: E. A. Perry. Fax: +1-202-673-4686; E-mail: eperry@athena.nwafc.nf.ca

1987; Boness 1990; Boness *et al.* 1992). Two forms of fostering are apparent: nursing other pups after becoming separated from one's own and nursing other pups simultaneously with one's own (simultaneous fostering). Simultaneous fostering is probably costlier than fostering a pup after the loss of one's own, as occurs in Hawaiian monk seals *Monachus schauinslandi* (Boness 1990), and is probably a result of one or more selective benefits that outweigh the high cost.

Fostering in grey seals appears to be relatively common. It typically consists of simultaneous fostering and its frequency varies among colonies (Kovacs 1987; D. J. Boness & E. A. Perry, unpublished observation). The combination of philopatry (e.g. Allen *et al.* 1995; D. J. Boness, unpublished data), breeding site fidelity by males and females (Pomeroy *et al.* 1994; Twiss *et al.* 1994), and evidence of mate fidelity (Amos *et al.* 1995) increase the likelihood that there is a higher degree of within-colony relatedness among grey seals in smaller colonies than in larger ones. Therefore, kin selection could play a role in the evolution of fostering behaviour in this species and account for differences in frequency among colonies.

This study investigates the potential for kin selection to play a role in the evolution of grey seal fostering behaviour. We compare the genetic similarity, based on DNA

fingerprints (Jeffreys *et al.* 1985a,b; Lynch 1990, 1991) between females and fostered pups to that between females and filial pups, and between unrelated individuals in a large breeding colony at Sable Island, Canada. In addition, we compare genetic similarity between fostered pups and the females' biological offspring to unrelated pups at two smaller colonies, Faray Island, Orkney, and Ramsey Island, Wales.

Materials and methods

Study sites

Our study was conducted at three grey seal breeding locations: (i) Sable Island, Canada (44 °N 60 °W), (ii) Faray Island, Orkney (59 °N 3 °W), and (iii) Ramsey Island, Wales (52 °N 5 °W). Sable Island, a sandbar in the northwestern Atlantic, provides a large habitat in which grey seals breed each January. Faray and Ramsey are smaller islands, in the north-eastern Atlantic, with isolated cliff-bound beaches on which females give birth from September to December.

Field methods

As part of a long-term study of grey seal maternal behaviour on Sable, branded females and their newborn pups were paint-marked within a day of birth in 1993 and 1994. During daily surveys, some of these branded females were found with other pups in addition to their own or only with other pups. Using a livestock earnotcher (Nasco), small skin samples were collected from females, their biological offspring (if available), and fostered pups, and in each year from a subsample of females that appeared to nurse their biological pups exclusively.

On three beaches at Faray and two at Ramsey all mothers with newborn pups were paint-marked remotely and observed daily for 2–10 h throughout lactation. All occurrences of fostering were recorded, noting the identity of participants. Because females at these two locations spent most of lactation in the water and left the beaches upon approach by humans, it was impossible to obtain skin samples from them. Skin samples were collected from all pups on each beach.

A total of 269 animals was sampled, including samples from five foster groups from Sable (1993, 1994), three foster-filial pup pairs from Faray (1993) and 10 foster-filial pup pairs from Ramsey (1994). Samples were stored in salt-saturated DMSO and frozen until analysed.

Laboratory methods

DNA extraction and fingerprinting protocols are similar to those described by Loew & Fleischer (1996).

Approximately 5 µg of DNA was digested to completion in an excess of *Hae*III and the resulting fragments electrophoresed for 48 h through 1% agarose gels. DNA was vacuum blotted onto nylon membranes and hybridized with radio-labelled 33.15 and 33.6 probes (Jeffreys *et al.* 1985a) at 58 °C overnight. Membranes were washed under stringent conditions and exposed to X-ray film at –70 °C for 2–14 days.

All fragments between 20 kb and about 2.8 kb were scored for individuals. No comparisons were made between gels. Fragments were numbered sequentially, beginning with the largest, such that fragments of different mobility were given different numbers. Fragments of the same mobility in different lanes were numbered identically. Pairwise band-sharing coefficients (S, Lynch 1990) were calculated for all individuals on each gel. Mothers, their biological offspring and the fostered pups were run in adjacent lanes to minimize scoring errors.

Because some females fostered more than one pup and some pups were fostered by more than one female, we had to exclude some foster pair comparisons to ensure that no individual was represented more than once in analyses. To increase sample sizes and determine our power to distinguish between levels of relatedness, we standardized our S data among populations by calculating relatedness values (r; eqn 22 in Lynch 1991) for each pairwise comparison, using the background S for each population. Means, standard deviations, and 95% confidence intervals (CI) were calculated from r-values combined across populations.

Results

Behaviour

The number of fostering females and length of time they fostered varied among the three locations (Table 1). On Sable, in 1993, two out of 48 paint-marked females (4%) followed throughout lactation fostered. One female fostered the same pup for at least 11 days while the other fostered for at least 10 days. In 1994 none of the 21 females followed throughout lactation fostered pups, but three incidentally noted foster groups were included in genetic analyses. Therefore, the overall incidence of fostering on Sable was low (2.9%). On Faray three out of 78 (3.8%) females observed fostered daily. The percentage of fostering females on Ramsey (28% of 25) was much higher than on the other two islands (χ^2 = 19.962, d.f. = 2, P < 0.01).

DNA analysis

Eight gels were run, including two for within-island comparisons on Faray and Ramsey. A mean of 18.1 (SD = 4.7, n = 123) 33.15 bands and 10.9 (SD = 2.8, n = 100) 33.6 bands

Table 1 The number of females nursing their own and other pups simultaneously and the number of females fostering after separation from their pups. Values in parentheses represent the range in minimum fostering duration (days)

	Sable Island	Faray Island	Ramsey Island
Fostered simultaneously	5 (3–11)	3 (1–4)	10 (1–7)
Fostered after separating	1 (3)	0	0

were scored per individual. The 33.15 probe S score, 0.329 (SD = 0.124), fell within expected ranges for outbred populations, but the 33.6 probe score was very high (S = 0.643, SD = 0.121), as has been found in grey seals previously (Amos *et al.* 1993). There was a low correlation between S for 33.15 and 33.6 (Pearson Correlation, $r = 0.147$, d.f. = 1, $\chi^2 = 9.619$, $P = 0.002$). In addition, mean S between presumed unrelated pups was significantly higher on Faray than either Sable or Ramsey, based on 33.15 fingerprints ($F_{2,433} = 5.499$, $P = 0.004$). In contrast, mean S values between unrelated pups on Faray and Sable, based on 33.6 fingerprints, were similar and both were significantly greater than that between pups on Ramsey ($F_{2,335} = 3.503$, $P = 0.031$). Therefore, data from the different probes were analysed separately.

We calculated r from our pairwise S-values (eqn 22, Lynch 1991) to standardize our data and allow us to combine data across populations, thereby increasing our power to detect differences. Mothers and biological offspring had r-values not significantly different from the 0.5 expected for first-degree relatives for both probes based on CI surrounding r (Table 2). Fostering females and fostered pups had lower mean r-values than biological pairs for

Table 2 Average relatedness values (r) and confidence intervals (CI) between Sable Island mothers, their biological offspring and their fostered pups, and between fostered and filial pups (offspring of the fostering females) pairs across all islands

	PROBE	
	33.15	33.6
Mother–Offspring	0.576	0.629
	CI: ± 0.082	CI: ± 0.148
	$n = 17$	$n = 11$
Mother–Foster pup	0.054	0.251
	CI: ± 0.079	CI: ± 0.099
	$n = 7$	$n = 5$
Foster–Filial pup	0.013	− 0.068
	CI: ± 0.09	CI: ± 0.12
	$n = 11$	$n = 8$

both probes (Table 2). Mean r-values for fostered and filial pups did not differ from 0 for both probes. All but the r-value calculated from 33.6 data for mothers and fostered pups are not different from the expected $r = 0$ for unrelated individuals. Based on CIs we would be able to detect relatedness between pups below the level of first cousins ($r = 0.125$) and between females and fostered pups to the level of first cousins.

To test for the possibility of philopatry leading to increased relatedness within small, isolated breeding beaches, we compared S of unrelated pups within and between beaches on Faray and Ramsey. There were no significant differences within locations (Table 3). Sable pups were excluded from the analyses as the breeding habitat is large with few topographical barriers to prevent the colony from being continuous.

Table 3 Average S (± SD) between presumed unrelated individuals within and between beaches on Faray and Ramsey Islands

	PROBE	
	33.15	33.6
Faray Island		
Beach 1	0.33 ± 0.14	0.79 ± 0.08
Beach 2	0.32 ± 0.07	0.68 ± 0.06
Beach 3	0.35 ± 0.09	0.72 ± 0.11
Between Beaches*	0.41 ± 0.09	0.76 ± 0.11
Ramsey Island		
Beach 1	0.30 ± 0.08	0.57 ± 0.19
Beach 2	0.31 ± 0.08	0.65 ± 0.13
Between beaches†	0.23 ± 0.03	0.55 ± 0.05

*33.15: $F_{3,16} = 0.885$, $P = 0.47$; 33.6: $F_{3,12} = 0.915$, $P = 0.46$.
†33.15: $F_{2,9} = 1.38$, $P = 0.29$; 33.6: $F_{2,9} = 0.624$, $P = 0.56$.

Discussion

Grey seal fostering has been anecdotally documented in several colonies of differing sizes. Evidence of grey seal philopatry (Allen *et al.* 1995; D. J. Boness, unpublished data), mate fidelity (Amos *et al.* 1995) and breeding-site fidelity (Pomeroy *et al.* 1994; Twiss *et al.* 1994) suggest that there could be increased relatedness of individuals on smaller breeding beaches than on larger ones. Thus, kin selection could operate as a selective force in fostering behaviour in this species. However, the results of our study suggest that this may not be the case.

On Sable, where we could compare the genetic similarity between fostering females and fostered pups, we found

relatedness values (*r*) similar to those between females and unrelated pups. Based on CIs surrounding relatedness values between females and fostered pups, we could detect differences to the level of first cousins (*r* = 0.125) using 33.15 and 33.6 fingerprints. Therefore, females could be caring for pups that were less related than first cousins.

Although we found a correlation between genetic similarity calculated from 33.15 and 33.6 fingerprints, background bandsharing was higher based on the 33.6 fingerprints. The 33.6 probe gave poor resolution and we had little confidence in those data. Amos *et al.* (1993) primarily relied on data from 33.15 fingerprints, using 33.6 to clarify grey seal paternity when necessary. The 33.6 probe may not be a useful tool to assess genetic similarity in grey seals.

On Faray and Ramsey Islands where we compared genetic similarity between fostered and filial pups to that of other pups on the beaches, there was no difference in relatedness between filial–foster pup pairs and unrelated pups on the same beaches, and we would have detected differences below the level of first cousins. Therefore, kin selection is an unlikely explanation for the fostering behaviour that we observed at any of the three colonies.

Philopatry, in combination with breeding-site fidelity and mate fidelity, increases the likelihood that breeding sites will contain relatives, a requisite for kin selection to contribute to fostering. We found no evidence to suggest that there was increased genetic similarity among pups born within various beaches on Faray or Ramsey compared to the level of genetic similarity between beaches. This apparent lack of variation in relatedness might help to explain why kin selection did not appear to be a selective force in the occurrence of fostering at these sites.

Other possible benefits to mammalian fostering behaviour have been proposed. Fostering could increase maternal experience for younger females that have lost their own offspring (Boness *et al.* 1992; Riedman 1982). Also, females that lose their pups before they become receptive at the end of lactation could, through fostering, ensure that they stay in the vicinity of breeding males (Riedman & LeBoeuf 1982). Although we have no direct data to address these possibilities, they seem unlikely explanations for the occurrence of fostering in grey seals as both of these benefits are dependent on females losing their own offspring before fostering pups. Our data indicate that grey seal females tend to foster simultaneously with nursing their own pups.

Our finding that female grey seals engage in simultaneous fostering is consistent with earlier findings (Kovacs 1987). Fostering of this nature should be costly in terms of reduced milk transfer to filial pups. This might explain the low fostering frequency on Sable and Faray, in which only 2.9% and 4% of observed females fostered, respectively. However, 28% of observed females on Ramsey fostered pups.

Higher female density and accompanying aggression, in combination with poor offspring recognition, increase fostering frequency in some seal species (Job *et al.* 1995; Riedman 1982; Boness *et al.*, in press). These factors cannot explain the high fostering frequency we observed on Ramsey because it had smaller beaches and lower densities than the other two islands. These conditions, however, in tandem with breeding-site fidelity might lead to greater short- and long-term familiarity among females such that the potential for reciprocity (see Riedman & LeBoeuf 1982) is greater. We do not have the data to address this possibility.

Female attendance patterns vary among colonies such that females on Sable remain on land with their pups throughout lactation while females in the smaller Faray and Ramsey colonies move from land to water between nursing bouts. These movements might increase the potential for fostering, especially if offspring recognition is poor.

Many questions remain to be answered before we understand why fostering occurs in grey seals. For example, we do not know how energetically costly fostering is, nor do we know whether filial offspring of fostering females suffer lower survival than those of nonfostering females. We might surmise lower survival because twinning in the wild is infrequent, suggesting constraints on the ability of females to rear more than one pup per season. In this study we did not quantify the amount of time females spent nursing foster pups compared to their own, nor did we obtain growth rates of pups. To understand whether and how fostering might be maintained at high levels by selective benefits, such data are needed to assess the costs. It is possible that fostering in grey seals does not produce any direct benefits that outweigh its costs but, rather, there are benefits to colonial breeding that compensate for the cost of fostering (cf. Wilkinson 1992; McCracken 1984). The close temporal links between rearing offspring and mating in grey seals, evidence of negative effects of male harassment on maternal care, and evidence of injuries to females by male sexual activity (LeBoeuf & Mesnick 1991; Boness *et al.* 1995) provide some basis for this argument.

Acknowledgements

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