Evidence for Mate Fidelity in the Gray Seal

Bill Amos,* Sean Twiss, Paddy Pomeroy, Sheila Anderson†

Colonially breeding gray seals are polygynous. Males are larger than females, compete with each other for position among aggregated females, and contribute no parental care. Genetic analysis of pups born on the island of North Rona, Scotland, reveals large numbers of full siblings, although dominant males father disproportionately few of these. This result cannot be explained by mating patterns based solely on male dominance and the spatio-temporal organization of the breeding colony. Instead, many full siblings must result from choices favoring previous parental combinations. Thus, polygyny and partner fidelity appear to operate simultaneously in this breeding colony.

In mammals, most of the reproductive costs are borne by the female, with males often contributing little more than sperm. This unequal investment has been used to explain why over 90% of mammals are polygynous (*1*), whereas monogamy is effectively restricted to the few species in which paternal care is important or in which females are widely dispersed (*2, 3*). With the advent of molecular genetic techniques capable of resolving close family relations, this predictive framework can be tested.

Our studies on the gray seal, *Halichoerus grypus*, reveal an unexpected mating pattern. This species has been described as polygynous (*2, 4, 5*). However, although some males increase their fitness by exerting dominance over other males (*6, 7*), many seals mate preferentially with previous partners. These contrasting behaviors appear to operate simultaneously in the same breeding colony.

Gray seals breed colonially at remote sites around the British Isles (*8*). In autumn, females come ashore for about 18 days to give birth to single pups, suckle, and mate. Mating usually occurs near the pupping site (*4, 9, 10*). Males come ashore for varying lengths of time, during which some compete aggressively for positions among the females (*5, 6*). At all colonies, females outnumber males.

On North Rona, a small cliff-bound island off northwest Scotland, approximately 1500 pups are born annually (*11*). Limited access and topographical barriers divide the breeding area into three effectively discrete regions: Study Area (SA), Fianuis South (FS), and Fianuis North/Central (*4, 12*). Colony sex ratio varies widely throughout the season but averages 7:1 (female:male) (*5, 6*). Field observations (1986 to 1989) focused on 85 males and 88 females marked after capture by unique brands. Capture for branding selected individuals who stood their ground, biasing our sample strongly toward dominant males (*6*). Branded females possibly suffer a similar bias.

Branded females show a mean annual return rate to North Rona of 67%, allowing two or more pups to be sampled from each of a number of females [that is, maternal half-siblings (half-sibs) (*12*)]. Using two methods, DNA fingerprinting (n = 39 pairs) and single-locus minisatellite analysis (n = 48 pairs), we examined paternal relations among such pups (Fig. 1). We estimate that 30% of all comparisons involve full siblings (full sibs). This figure equates to a surprising degree of mate fidelity, with the precise degree depending on male reproductive longevity (r) (*13*). Substituting r = 10 years, we find that 59 to 100% of an average female's pups are fathered by only 1 to 2.9 males. Smaller values of r imply a more polarized pattern of mating, with fewer males accounting for more of each female's pups. Larger values of r change the predictions little and are probably unrealistic (*13*).

How can parental combinations recur so frequently? There are two possible mechanisms. Either a female's mate is determined solely by the relative proximity and dominance of neighboring males and colony organization changes little between seasons, or seals recognize and select previous partners. Because many potential fathers cannot be sampled (*14*), these two alternatives cannot be distinguished by direct paternity analysis. However, the first possibility can be tested by use of detailed field observations to determine how frequently full sibs should arise by chance.

If females mate usually with the nearest male, full sibs are likely to be born only to parents who both return to similar locations within the colony. Females and males show similar degrees of site fidelity [median between-season displacements: 55 m and 53 m, respectively (*6, 12*)]. To examine this on

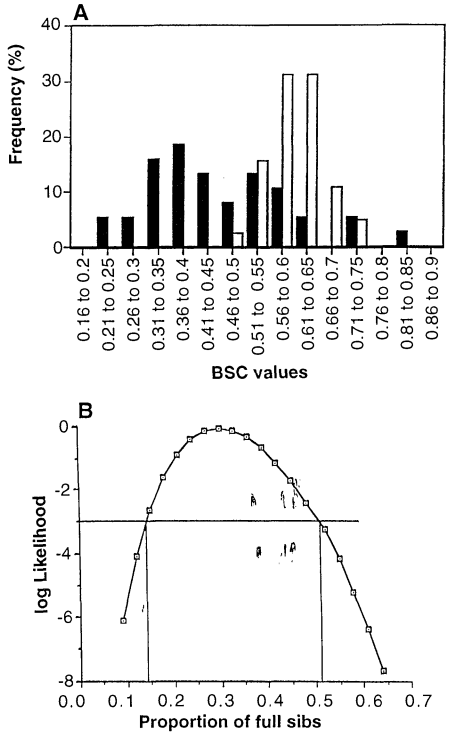

Fig. 1. Determination of the frequency of full sibs in comparisons between maternal half-sibs. Method 1: Adjacent-lane DNA fingerprint band-sharing coefficients (BSC) of relatedness (*7*) were derived for 89 mother-pup pairs (white bars, mean = 0.61, SD = 0.07) and 39 pairs of maternal half-sibs (black bars) (**A**). Unrelated BSC values were determined with the use of 70 adult males (mean = 0.25, SD = 0.06). All possible comparisons between half-sibs could not be made because some samples were inadequate in either quality or quantity. Half-sib BSC values cover the full range of relatedness, from unrelated to full sib. Assuming BSC values are distributed approximately normally, the probability of obtaining the observed distribution of half-sib BSC values was calculated for all possible combinations of full sibs, half-sibs, and unrelated pups. The half-sib BSC distribution was assumed to have a mean and variance intermediate between those for unrelated and mother-pup pairs. Method 2: Forty-eight pairs of maternal half-sibs were typed for the hypervariable seal minisatellite locus *HgMS-A5* [gene identity (G) = 0.032, 57 alleles recorded (*7*)]. Paternally unrelated pups share paternal bands with probability = G. Full sibs share paternal alleles with probability = 0.5 (1 + G) = 0.52. Using these values, we calculated the probabilities of observing each of all possible proportions of full-sibs using a standard binomial expansion. Probability values from methods 1 and 2 were then combined into a single log likelihood curve (**B**). The combined best estimate is 30% full sibs (95% confidence interval = 14.5% – 51%, taken as three log units on either side). Extrapolating back to our entire data set of 120 pups, 21 pairs of full sibs are expected. This method does not identify specific pairs with confidence.

B. Amos, Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH, UK.
S. Twiss, Department of Biological Sciences, University of Durham, South Road, Durham, DH1 3LE, UK.
P. Pomeroy and S. Anderson, Sea Mammal Research Unit, High Cross, Madingley Road, Cambridge, CB3 OE7, UK.

*To whom correspondence should be addressed.
†Present address: Doirlinn View, Church Brae, Tobermory, Isle of Mull, PA75 6PH, UK.

an individual basis, we generated snapshot maps for the positions of all males in SA on three dates (the estimated peak of estrus and 1 week on either side) in each of three seasons from 1987 to 1989 62.3% of males appeared in snapshots from only one season, making them unlikely to father full sibs through site fidelity. The remaining, resighted males were all branded. Among these, several showed site fidelity that was sufficient to allow repeat pairings with nearby females who were site faithful (Fig. 2).

Branded males are also more likely to father full sibs on the basis of their temporal distribution. Estrus dates for individual females vary little between seasons (*15*). Consequently, only males who are present on or around the same date in two or more seasons are likely to father full sibs. During 1987 to 1989, 252 males were recorded ashore in SA (seasonal average 108). Of these, only 41 (16%) showed any temporal overlap (Fig. 3), and branded males accounted for 96% of the total score.

Thus, the distribution of adult seals in space and time predicts that the vast majority of full sibs will be fathered by branded males. This is expected because branded males are significantly more dominant than unbranded males and enjoy greater average reproductive success (*6*, *7*). However, branded males actually father disproportionately few full sibs (*16*). In the absence of individual-based mate choice, these observations can be rationalized only if the few unsampled males that spend substantial amounts of time in the breeding colony are highly successful. If such males exist, our sample of pups should reveal high overall degrees of shared paternity. We therefore used diversity among paternal alleles at hypervariable minisatellite locus *HgMS-A5* to estimate the mean within-season probability of shared paternity (*17*). The resulting value, 0.023, denies the existence of highly successful males and can explain only 1 in 13 of the full sibs we observed.

If most full sibs are born to diverse unbranded males, we must conclude that many pairs of seals establish durable ties, recognizing each other between seasons and coordinating their behaviors. Such a pattern may have been overlooked in previous studies, first, because it occurs alongside the more obvious behaviors associated with polygyny and, second, because it is inherently difficult to identify interseason links between many specific, often brief interactions. Despite this, a number of field observations are consistent with a preference for previous partners (Table 1).

Many aspects of gray seal breeding biology favor polygyny. Therefore, the existence of widespread partner fidelity implies that an important component of individual fitness has been overlooked. A plausible candidate is pre-weaning pup mortality, which varies greatly with time and locality but can reach 60% (*18*). A significant proportion of this mortality can be ascribed to disturbances caused by aggressive interactions involving males (*19*). Partner fidelity should reduce these disturbances and therefore increase pup survival rates. However, the mechanism by which the observed pattern could evolve as the consequence of individually favored mating strategies remains unclear.

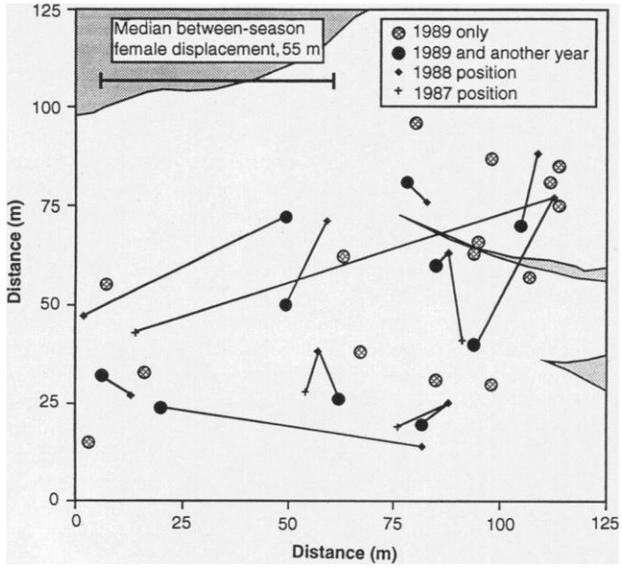

Fig. 2. Male site fidelity. The positions of all adult males in SA were recorded on three dates (at the peak of estrus and 1 week on either side) in each of three seasons (1987 to 1989) (*6*). The map covers an area used by approximately 90% of the females in SA; distances are in meters and shaded areas are sea. Data represent the estimated peak of estrus (16 October) in 1989. The scale bar is included to help the reader visualize how infrequently an average female will be next to the same male in consecutive seasons.

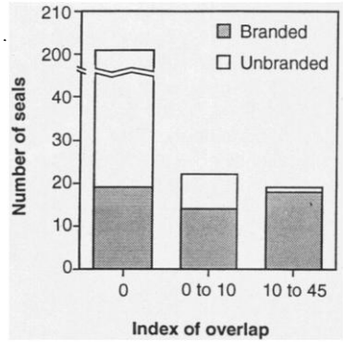

Fig. 3. Duration and timing of stay of males within the breeding colony: the degree of between-season overlap. Intensive observations were made daily during daylight hours (8 hours) for the breeding seasons from 1987 to 1989 (*20*). All males who came ashore in SA for more than 1 hour were identified by either letter-number brands or natural markings (*6*, *20*). Absences of 2 days or less were ignored. Mean stay ashore in SA was 20.1 days for branded males and 5.1 days for unbranded males. Indices of temporal overlap were calculated as follows: 1 for each day in common between any two seasons and 0.5 for days not in common but lying within 2 days of each other. A 0 score thus indicates presence in one season only or nonoverlapping stays in two or more seasons. All daily scores were adjusted by a weighting according to the relative number of estrous females available (derived from parturition data for all females in SA) and normalized to give a maximum score of 1 per day.

Table 1. Behavioral observations supporting the existence of partner fidelity.

Behavior	Description
Long-term association and coordinated movement	Seven years after fathering her pup, male S2 was seen attending female J8 400 m away in a different island subregion. Positional information is available for five mothers of probable full sibs. Two were site faithful, but three moved >120 m between seasons. Mothers of two pairs of highly probable full sibs were not seen on North Rona in the year of conception of one of the pups. Paternity testing has revealed three instances in which one parent was not seen on North Rona in the year of conception (*7*). Occasionally, mating is observed in the shallows around the colony.
Female mate choice	Although females rarely show overt signs of mate solicitation, they frequently reject male advances (*21*).

REFERENCES AND NOTES

1. T. H. Clutton-Brock, *Proc. R. Soc. London Ser. B* **236**, 339 (1989).
2. S. T. Emlen and L. W. Oring, *Science* **197**, 215 (1977).
3. T. H. Clutton-Brock and P. H. Harvey, *Nature* **273**, 191 (1978).
4. J. M. Boyd, J. D. Lockie, H. R. Hewer, *Proc. Zool. Soc. London* **138**, 257 (1962).
5. S. S. Anderson, R. W. Burton, C. F. Summers, *J.*

Zool. (London) **177**, 179 (1975).
6. S. D. Twiss, P. P. Pomeroy, S. S. Anderson, *ibid.* **233**, 683 (1994).
7. W. Amos, S. S. Twiss, P. P. Pomeroy, S. S. Anderson, *Proc. R. Soc. London Ser. B* **252**, 199 (1993).
8. W. N. Bonner, *Oceanogr. Mar. Biol. Annu. Rev.* **10**, 461 (1972).
9. H. R. Hewer, *Proc. Zool. Soc. London* **142**, 593 (1964).
10. J. C. Coulson and G. Hickling, *J. Anim. Ecol.* **33**, 485 (1964).
11. A. R. Hiby, C. D. Duck, D. Thompson, *NERC News* (January 1993), p. 20.
12. P. P. Pomeroy, S. S. Anderson, S. D. Twiss, B. J. McConnell, *J. Zool. (London)* **233**, 429 (1994).
13. For any female, the probability of a pair of her pups being full sibs (F) relates to the probability of paternity by all n possible males as follows:

$$F = \sum_{i=1}^{n} P_{i1}P_{i2}$$

where P_{i1} and P_{i2} are the probabilities of the i^{th} male fathering pups 1 and 2, respectively. If P_{i1} and P_{i2} differ principally by the probability that the male stops reproducing between the two conceptions, $P_{i1}P_{i2}$ can be rewritten as

$$P_i^2\left(1 - \frac{a}{r_i}\right),$$

where a is the time interval separating the two pups and r_i is the reproductive longevity of the i^{th} male. Substituting mean values for a and r, F = 0.3 and rearranging gives:

$$\frac{0.3\,\bar{r}}{(\bar{r} - \bar{a})} = \sum_{i=1}^{n} p_i^2$$

For our data set, $\bar{a}$ = 1.53. Male reproductive longevity is unknown. Bonner (*8*) suggests that 90% of males die by age 15 and that none live beyond 20. Yet, 25 branded males (~30%) were resighted in a single season 7 years later. Because these males were mature at branding, and few males reappear every year, we suggest that r = 10 is a reasonable value. Substituting and solving for P_i, we can identify two extremes. If a single male is likely, all others being highly unlikely, he will father 59% of the female's pups during his lifetime. If all fathers are equally likely, there must be 2.86 males who between them father 100% of her pups.
14. Sampling is limited by lack of opportunity and the need to minimize disturbance.
15. Estrus is taken as 14 days post-parturition [I. L. Boyd, *J. Reprod. Fertil.* **69**, 157 (1983)]. The mean between-season shift of 2.54 days (±0.3 SE) was observed for 59 females in our data set. Pupping is initially late, becoming progressively earlier with age (*12*).
16. Paternity data are from (*7*). Seventy branded males were tested against 120 pups that included an estimated 21 pairs of full sibs. Branded males gained 29 paternities but only two pairs of full sibs: $\chi^2 = 7.56$, $P < 0.01$.
17. The mean probability of shared paternity, P_s, among a sample of pups can be estimated from the difference between the population gene identity (G, see legend to Fig. 1) and the gene identity calculated for paternal alleles among pups, G_p, as follows:

$$G_p = G(1 - P_s) + \frac{P_s(1 + G)}{2}$$

and, by rearrangement:

$$P_s = \frac{2(G_p - G)}{(1 - G)}$$

Within-season values of G_p were calculated for 84 pups born to branded females, half-alleles being assigned in cases in which the paternal allele was ambiguous (1986, n = 16, 12.5 alleles, G_p = 0.063; 1987, n = 25, 18 alleles, G_p = 0.053; 1988, n = 18, 15.5 alleles, G_p = 0.022; and 1989, n = 24, 20 alleles, G_p = 0.035), giving a weighted mean of 0.043 and an overall P_s of 0.023.
18. C. F. Summers, R. W. Burton, S. S. Anderson, *J. Zool. (London)* **175**, 439 (1975).
19. J. R. Baker, *ibid.* **203**, 23 (1984); B. J. Le Boeuf, in *Behaviour of Pinnipeds*, D. Renouf, Ed. (Chapman & Hall, London, 1991), pp. 45–65; _____ and K. T. Briggs, *Mammalia* **41**, 167 (1977).
20. S. D. Twiss, thesis, University of Glasgow (1991).
21. D. J. Boness, S. S. Anderson, C. R. Cox, *Can. J. Zool.* **60**, 2270 (1982).
22. We thank P. Clapham, T. Clutton-Brock, G. Cooper, C. Duck, M. Fedak, J. Harwood, and J. Pemberton for comments on the manuscript; J. A. Barrett for statistical advice; I. A. van Pijlen for cloning locus *HgMS-A5*; and many colleagues for help during fieldwork. S.T. was supported by a National Environment Research Council (NERC) CASE studentship; B.A. was supported by NERC and the Royal Society.

16 November 1994; accepted 5 May 1995

Common Virulence Factors for Bacterial Pathogenicity in Plants and Animals

Laurence G. Rahme, Emily J. Stevens,* Sean F. Wolfort, Jing Shao,† Ronald G. Tompkins, Frederick M. Ausubel‡

A *Pseudomonas aeruginosa* strain (UCBPP-PA14) is infectious both in an *Arabidopsis thaliana* leaf infiltration model and in a mouse full-thickness skin burn model. UCBPP-PA14 exhibits ecotype specificity for *Arabidopsis*, causing a range of symptoms from none to severe in four different ecotypes. In the mouse model, UCBPP-PA14 is as lethal as other well-studied *P. aeruginosa* strains. Mutations in the UCBPP-PA14 *toxA, plcS, and gacA* genes resulted in a significant reduction in pathogenicity in both hosts, indicating that these genes encode virulence factors required for the full expression of pathogenicity in both plants and animals.

Bacterial pathogens comprise a large and diverse group of species capable of infecting both animals and plants. Most of these pathogens cause disease in a single or limited number of host species. The interactions between bacterial and host factors that limit host range and determine resistance or susceptibility are not fully understood.

Despite the vast evolutionary gulf between plants and animals, two types of observations suggest that some of the underlying mechanisms of bacterial pathogenesis may be similar in the two kingdoms. First, bacterial proteins involved in the export of proteinaceous virulence factors have been shown to be conserved between plant and mammalian pathogens (*1*). Second, for some bacterial species, including *Pseudomonas cepacia* (*2*), *Pseudomonas aeruginosa* (*3*, *4*), and *Erwinia* spp. (*5*), specific strains have been reported to be either plant or animal pathogens.

Reports indicating similarities between plant and animal pathogens prompted us to search for a strain of *P. aeruginosa* that was capable of eliciting disease in both a well-defined plant pathogenesis model and a well-defined animal pathogenesis model. We chose *P. aeruginosa* for these studies because it is a serious opportunistic pathogen in immunocompromised human patients (*6*) and because individual clinical isolates have been reported to cause disease in plants (*3*). Given such a "dual" animal-plant pathogen, it would be interesting from an evolutionary perspective to determine which, if any, bacterial virulence factors were involved in both plant and animal pathogenesis.

A collection of 75 *P. aeruginosa* strains (*7*), of which 30 were human isolates, were screened for their ability to cause disease on leaves of at least four different *Arabidopsis thaliana* ecotypes (*8*, *9*) (land races or wild accessions). We reasoned that a *P. aeruginosa* pathogen that exhibited ecotype specificity on *Arabidopsis* would most likely be a true plant pathogen, rather than a strain that has no capacity to be a plant pathogen under natural settings but infects plants as a consequence of the artificial environment created in the laboratory (*10*).

Most of the 75 *P. aeruginosa* strains that were screened elicited no symptoms in *Arabidopsis* leaves. Several strains elicited weak to moderate soft-rot symptoms. However, two strains, UCBPP-PA14, a human isolate, and UCBPP-PA29, a plant isolate, caused severe soft-rot symptoms in some, but not all, of the ecotypes tested, a result

L. G. Rahme and F. M. Ausubel, Department of Genetics, Harvard Medical School, Boston, MA 02114, USA, and Department of Molecular Biology and Shriners Burns Institute, Massachusetts General Hospital, Boston, MA 02114, USA.
E. J. Stevens, Shriners Burns Institute, Massachusetts General Hospital, Boston, MA 02114, USA.
S. F. Wolfort and R. G. Tompkins, Shriners Burns Institute, Massachusetts General Hospital, Boston, MA 02114, USA, and Department of Surgery, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114, USA.
J. Shao, Department of Genetics, Harvard Medical School, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA.

*Present address: National Institutes of Health, National Cancer Institute, Surgery Branch, Rockville, MD 20892, USA.
†Present address: Millennium Pharmaceutical, Cambridge, MA 02139, USA.
‡To whom correspondence should be addressed.