GENETIC ANALYSIS OF TWINNING IN ANTARCTIC FUR SEALS (*ARCTOCEPHALUS GAZELLA*)

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Twinning in natural pinniped populations is often inferred from observations of suckling behavior, but this approach has been criticized because nonfilial nursing occurs at high frequencies in many seal species. Consequently, we used 9 highly polymorphic microsatellite markers to examine the parentage of 11 putative pairs of twins in Antarctic fur seals (*Arctocephalus gazella*) breeding at Bird Island, South Georgia. Only 3 pairs (27%) were found to be genuine twins, indicating that suckling observations are an unreliable means of identifying twins in this species. All of the twins were female; 1 pair was monozygotic and the other 2 were dizygotic. Using a strict exclusion approach, paternity was assigned to the monozygotic but not the dizygotic twins. However, likelihood tests revealed that, of the latter, 1 pair was significantly more likely to be full siblings against the null of half sibship suggesting shared paternity, whereas the other pair was more likely to be half siblings against the null of full sibship indicating probable multiple paternity. Our results provide novel insights into the reproductive ecology of fur seals and also support an earlier study showing that molecular genetic analysis can provide an effective means of validating field observations of pinniped twins.

Key words: dizygotic twins, fostering, maternal investment, microsatellite, monozygotic twins, paternity, pinniped, otariid, sperm competition, twin

Twinning is infrequent in pinnipeds (accounting for only 0.1–0.4% of births [Fay et al. 1991; Gelatt et al. 2001; McMahon and Hindell 2003]) and twin births are rarely sighted (Spotte 1982). Consequently, it has become common practice to infer twinning from field observations of suckling behavior (e.g., Arnbom et al. 1997; Bester and Kerley 1983; Doidge 1987; Haase 2007). This approach is relatively easy to implement and provides an appealing alternative to traditional destructive methods, but has been criticized (McMahon and Hindell 2003; Spotte 1982) because foster nursing is widespread among pinnipeds, reaching frequencies of up to 90% (e.g., Boness et al. 1998; Lunn 1992; Perry et al. 1998; Stirling 1975).

Fortunately, molecular methods, and in particular microsatellites, offer a convenient solution to this problem. Microsatellites are DNA segments comprising tandem repeats of 1–6 nucleotides that occur abundantly in the nuclear genomes of most eukaryotes and have a sufficiently high mutation rate to generate and maintain extensive polymor-

phism (Tautz and Renz 1984). They can be readily amplified using the polymerase chain reaction (Saiki et al. 1985) from a variety of tissues including shed skin and hair (Caudron et al. 2006; Swanson et al. 2006) and also typically follow a Mendelian pattern of inheritance, making them ideally suited to parentage analysis.

Importantly, microsatellites can be used not only to verify that twins share the same mother, but also to assign paternity (Gelatt et al. 2001), with the potential to yield insights into the mating system of the species under study. For example, reports of human dizygotic twins with different fathers (e.g., Verma et al. 1992; Wenk et al. 1992) provide indisputable genetic evidence for partner infidelity. Similarly, multiple paternity has also been documented in twin rhesus macaques (*Macaca mulatta*—Bercovitch et al. 2002), white-tailed deer (*Odocoileus virginianus*—Sorin 2004), and big brown bats (*Eptesicus fuscus*—Vonhof et al. 2006), supporting the widely held view that multiple mating and sperm competition could be a feature of the mating system of many mammalian species.

Pinnipeds are interesting in the above context because they exhibit a broad range of mating systems, from aquatic mating and low reproductive skew in harbor seals (*Phoca vitulina*—Coltman et al. 1998), hooded seals (*Cystophora cristata*—McRae and Kovacs 1994), and Weddell seals (*Leptonychotes*

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weddellii—Harcourt et al. 2008) to terrestrial mating and strong polygyny in southern elephant seals (*Mirounga leonina*—Hoelzel et al. 1999), Antarctic fur seals (*Arctocephalus gazella*—Hoffman et al. 2003), and northern fur seals (*Callorhinus ursinus*—Kiyota et al. 2008). Moreover, females of several pinniped species are known to copulate with multiple partners within breeding seasons (Ambs et al. 1999; Fabiani et al. 2004; Hoffman et al. 2003; Perry and Amos 1998), raising the possibility that sperm competition could be important in these, and potentially in other seal species. Interestingly, the only genetic study published to date of twinning in a pinniped species (Gelatt et al. 2001) found no evidence for multiple paternity in a natural population of Weddell seals. However, with a sample size of only 3 pairs of twins in a species that is only mildly polygynous (Harcourt et al. 2008), the chance of finding 1 or more cases of multiple paternity would appear to be small.

The Antarctic fur seal provides an ideal opportunity to study twinning in a natural pinniped population. This species is sexually dimorphic, exhibiting resource-defense polygyny and highly synchronous seasonal breeding (Bonner 1968). At Bird Island, South Georgia, a breeding colony has been studied since the 1950s. Access to the colony is provided by an aerial walkway (Doidge et al. 1984) that allows much greater ease of observation, marking, and tissue sampling than at many other field sites. Tissue samples have now been collected for almost a decade, and the use of a panel of 9 hypervariable microsatellite markers allows both maternity and paternity to be assigned to pups with high confidence (Hoffman et al. 2003). Each austral summer, pregnant female Antarctic fur seals haul out on breeding beaches during late November to mid-December to give birth to pups conceived the previous season. They remain ashore to suckle their pups for 5–7 days, then mate and return to sea to feed. Foraging trips last 3–7 days and alternate with 1- to 2-day shore visits in which the young are suckled. Finally, after a lactation period of approximately 120 days, the pups are weaned (Doidge et al. 1986).

Previously at the Bird Island colony, Doidge (1987) identified 2 pairs of females that were each observed suckling 2 pups. One of the pairs of pups had wet fur indicating that they had probably been born within a few hours of each other. However, a definitive conclusion as to the filial status of these pups could not be reached because the births were not observed nor the placentae found. Moreover, nonfilial suckling was subsequently found to be widespread at the study colony (Hoffman and Amos 2005a; Lunn 1992) implying that suckling observations should be interpreted with caution.

Here, to determine unequivocally whether twinning occurs in Antarctic fur seals, we conducted a genetic analysis of 11 putative mother–twin triads identified during 9 consecutive field seasons. Microsatellite polymorphisms were used to determine whether these pups were genuine twins and a paternity analysis was conducted using both paternal exclusion and log-likelihood testing approaches.

MATERIALS AND METHODS

This study was conducted at Bird Island, South Georgia (54°00′S, 38°02′W), during the austral summers of 1994–1995 through 2002–2003 (hereafter, breeding seasons are referred to by the year in which they began). The population was located at a small cobblestone breeding beach covering an area of approximately 440 m^2 at high tide and with an average annual pup production during the study of 649 (Forcada et al. 2005). Adult females were identified using plastic tags (Dalton Supplies, Henley-on-Thames, United Kingdom) placed in the trailing edge of the foreflipper and a tissue sample was taken from the interdigital margin of the foreflipper using piglet ear-notching pliers. Pups born to tagged females were marked with temporary serial numbers by bleaching the fur on their backs and were tissue sampled in the same way as adult females. Adult males were given individually distinctive paint markings and sampled using a biopsy dart system. All tissue-sampling equipment was cleaned using ethanol between uses. Samples were stored individually in the preservative buffer 20% dimethylsulfoxide saturated with salt and stored at −20°C.

Total genomic DNA was extracted using an adapted Chelex 100 protocol (Walsh et al. 1991) and microsatellite genotyping was conducted as described in detail by Hoffman and Amos (2005b). Reactions yielding uncertain genotypes (e.g., with faint or unclear bands) were repeated, and consequently the genotyping error rate was very low, estimated at between 0.0013 and 0.0074 per single-locus polymerase chain reaction. The loci did not deviate significantly from Hardy–Weinberg equilibrium and were unlinked (Hoffman et al. 2003, 2006).

Each season, twice-daily surveys were made of the study beach from November 1 until the end of the pupping period (early January). Putative mother–twin triads were identified by the presence of 2 recently born pups with wet fur that were either observed suckling or were in close proximity to a single adult female. For each of these triads, we recorded the identity of all 3 individuals, together with the sex and birth weight of the 2 pups. In addition, whenever a pup died we recorded the date and conducted a gross necropsy (Hoffman et al. 2006) to determine the most likely cause of death.

To test whether the putative mother–twin triads identified in the field were genuine, we compared the adult female's genotype with that of the 2 pups. Following Gelatt et al. (2001), we calculated for each pup the probability of parent–offspring exclusion (*P[POE]*), given as the probability that a randomly selected individual from the population would be rejected as the parent. This was calculated over all 9 loci as the product of $(1 - p)^2$ for each locus where the pup was homozygous and p was the frequency of the homozygous allele and $(1 - q - r)^2$ for each heterozygous locus where q and r were the frequencies of heterozygous alleles. These calculations employed allele frequencies derived from 1,277 pups (21.6% of all pups born during the study), 87 adult females, and 464 adult males (total $n = 1,828$) sampled during the study period. The *P(POE)* was sufficiently low (Table 1, mean $P[POE] = 4.35 \times 10^{-4}$) that pups found to genetically

TABLE 1.—Summary of field observations and genetic analysis of 11 Antarctic fur seal (*Arctocephalus gazella*) putative mother–twin triads sampled during 1994–2002 and genotyped at 9 highly polymorphic microsatellite loci. Paternity was only tested for pups that genetically matched their putative mothers. *P(POE)* = probability of parent–offspring exclusion (see "Materials and Methods"). Note: Maternal relatedness values differ slightly for the monozygotic twins due to 2 missing single-locus genotypes (see Table 2). NS = not significant.

		Pup					Number of mismatching loci	$P(POE)$	Relatedness to putative mother	Relationship		
Year	Putative mother's ID	Pup's ID	Sex	Birth weight (kg)	Identity of father	Cause of mortality				Type of twin	Likelihood ratio (full versus half siblings)	Likelihood ratio (half versus full siblings)
1994	W5128/29	AGP94078	Male	4.7	—	—	6	2.86×10^{-4}	−0.231			
		AGP94079	Male	5	—	Starvation	0	1.66×10^{-3}	0.554			
1995	W3709/10	AGP95145	Female	4.5	—	Trauma	4	2.36×10^{-4}	−0.060			
		AGP95153		—	AGM94003	—	0	2.09×10^{-6}	0.489			
1998	W1222/23	AGP98002	Male	5.6	—	—	0	6.60×10^{-7}	0.540			
		AGP98049	Male	4.6	—	Unknown	4	2.21×10^{-4}	−0.103			
1998	W5623/24	AGP98006	Female	4.8	AGM95144	—	0	1.19×10^{-6}	0.457			
		AGP98099	Female	4.5	—	—	3	1.59×10^{-3}	0.304			
1999	W5049/50	AGP99044	Male	4.7	AGM98006	—	0	1.57×10^{-3}	0.556			
		AGP99045	Male	5.6	—	—	4	2.78×10^{-4}	−0.003			
2000	W6232/33	AGP00059	Male	5.2	—	—	4	2.17×10^{-4}	0.033			
		AGP00065	Female	5.3	—	Unknown	0	1.51×10^{-4}	0.184			
2000	W6264/65	AGP00092	Female	3.7	—	Unknown	0	1.26×10^{-6}	0.492	Dizygotic	3.02×10^{4} ($P < 0.001$)	3.32×10^{-5} ($P =$ NS)
2000	W6407/07	AGP00093	Female	4.3	—	—	0	2.13×10^{-6}	0.426			
		AGP00125	Male	6.0	AGM99097	—	0	1.63×10^{-6}	0.410			
		AGP00126	Female	5.5	—	—	3	7.92×10^{-5}	0.197			
2000	W5641/42	AGP00137	Female	3.7	AGM99129	Starvation	0	4.11×10^{-6}	0.425			
		AGP00139	Female	4.5	—	Unknown	4	8.35×10^{-7}	−0.130			
2000	W5006/07	AGP00491	Female	5.1	—	—	0	4.33×10^{-4}	0.547	Dizygotic	0.0743 ($P =$ NS)	13.45 ($P < 0.05$)
2002	W6287/88	AGP00492	Female	5.2	—	—	0	4.71×10^{-6}	0.565			
		AGP02113	Female	5.7	AGM00137	—	0	1.71×10^{-3}	0.521	Monozygotic	7.40×10^{3} ($P < 0.001$)	1.35×10^{-4} ($P =$ NS)
		AGP02114	Female	5.5	AGM00137	—	0	9.66×10^{-4}	0.503			

match the female (e.g., the 2 genotypes share a common allele at all of the loci genotyped) were classified as being filial, whereas those mismatching at 1 or more loci were classified as being nonfilial.

A paternal exclusion analysis was conducted as described in detail by Hoffman et al. (2003). Briefly, we screened each pup against all 464 sampled adult males, assigning paternity only when the candidate father matched the pup at all 9 loci, or where a single mismatch could be ascribed to a scoring error. To maximize confidence in our paternity assignments, only pups that genetically matched their putative mothers were analyzed. Where paternity could not be assigned to twins, we used log-likelihood tests implemented in the program Kinship 1.3.1 (Goodnight and Queller 1999) to distinguish full from half siblings ($R_m = 0.5$, $R_p = 0.5$ and $R_m = 0.5$, $R_p = 0$, respectively, where R_m is the probability of pups sharing an allele by maternal ascent and R_p is the probability of their sharing an allele by paternal ascent). Kinship reports likelihood values for a primary versus a null hypothesis, both of which are specified by the user. A high likelihood value favors the primary over the null hypothesis. The program also calculates *P*-values associated with the significance of the likelihood ratio through simulation (1,000 replicates were specified).

This work was conducted according to guidelines approved by the American Society of Mammalogists (Gannon et al. 2007) and also was approved by the British Antarctic Survey and the University of Cambridge Animal Ethics Board. Samples were collected and retained under permits issued by the Department for Environment, Food, and Rural Affairs and in accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

RESULTS

During 1994–2002, we identified 11 putative mother–twin triads. The probability of parent–offspring exclusion (*P[POE]*) was calculated with high precision for each pup using allele frequency data from 1,828 individuals genotyped during the study. The resulting values were very low ($\bar{X} = 4.35 \times 10^{-4}$, range = 6.60×10^{-7} to 1.71×10^{-3}), indicating that a randomly selected individual from the population would be highly unlikely to match a pup by chance.

Field observations and genetic analyses of the 11 putative mother–twin triads are summarized in Tables 1 and 2. In every case, the adult female was found to match at least 1 of the putative twin pups at all of the microsatellite loci that successfully amplified. However, only 3 females (27%) genetically matched both pups, indicating that twinning occurs at a lower frequency in this population than predicted from field observations alone.

One of the pairs of twins was monozygotic, whereas the other 2 were dizygotic (Table 1). Using a strict exclusion approach, paternity of the monozygotic twins was assigned to a male holding a territory approximately 2 body lengths distant from the mother on the day that the pup was conceived

(Hoffman et al. 2007). Unfortunately, it was not possible to assign paternity to either of the dizygotic pairs of twins. Consequently, we examined the paternal relatedness of these pups by conducting log-likelihood tests using Kinship 1.3.1 (Goodnight and Queller 1999). One pair had a significantly greater likelihood of being full siblings against the null of half sibship (likelihood ratio = 3.02×10^4, $P < 0.001$; Table 1), suggesting that they shared the same father. In contrast, the 2nd pair of twins was significantly more likely to be half siblings against the null of full sibship (likelihood ratio = 13.45, $P < 0.05$; Table 1), implying probable multiple paternity.

All 3 of the genetically confirmed pairs of twins were female. Of these pups, 5 had birth weights falling within the central 95% of the range reported for female pups at this colony (3.8–5.9 kg, $n = 540$ [Hoffman et al. 2006]) and survived to the end of the pupping period. The remaining pup weighed only 3.7 kg at birth (24.5% lighter than average) and died at 6 days of age. Although the necropsy was inconclusive, this pup most likely died from starvation or trauma, the primary causes of neonatal mortality at this colony (Hoffman et al. 2006; Reid and Forcada 2005).

DISCUSSION

Several authors have questioned the validity of using suckling observations to study twinning in natural pinniped populations (e.g., McMahon and Hindell 2003; Spotte 1982). However, to our knowledge only Gelatt et al. (2001) have attempted to verify the presence of twins genetically in a pinniped species, and no evidence has yet been reported for multiple paternity in seal twins. Consequently, we used microsatellites to examine twinning in an intensively studied population of Antarctic fur seals breeding at Bird Island, South Georgia.

Nonfilial suckling.—As found previously in Weddell seals (Gelatt et al. 2001), examination of our data shows that twinning cannot be accurately determined from field observations of suckling behavior in Antarctic fur seals, with only 3 (27.3%) of 11 putative pairs of twins being genuine. This finding is broadly consistent with previous studies of nursing behavior at the study colony. For example, Lunn (1992) used detailed daily behavioral observations to document almost 100 cases of nonfilial nursing during a single season, and in a subsequent genetic study, Hoffman and Amos (2005a) found that almost 10% of presumed mother–offspring pairs sampled over 8 consecutive years possessed incompatible genotypes. In the context of our current findings, it would be interesting to know whether the nonfilial pups involved were being fostered, implying that the triads were stable over time, or alternatively whether the pups were briefly engaged in milk-stealing. However, the latter possibility seems most likely given that Lunn (1992) attributed 68% (73 of 107) of all suckling observations at the study colony to milk-stealing.

Molecular genetic analysis was instrumental in refining the estimate of Gelatt et al. (2001) of the twinning rate in Weddell

TABLE 2.—Multilocus genotypes (sizes in base pairs) of 11 Antarctic fur seal (*Arctocephalus gazella*) putative mother–twin triads sampled during 1994–2002. In each case, the mother is listed before the 2 pups. — = no data.

Year	Animal ID	Type of twin	Locus Aa4	Locus Hg1.3	Locus Hg6.3	Locus Hg8.10	Locus Lw10	Locus M11a	Locus PvcA	Locus PvcE	Locus Pv9
1994	W5128/29		206 216	256 258	231 245	166 166	110 140	174 184	137 143	128 144	170 176
	AGP94078		216 218	254 254	233 245	164 164	128 130	154 184	145 151	124 130	168 168
	AGP94079		206 208	258 260	233 245	166 166	140 140	174 184	143 149	132 144	166 170
1995	W3709/10		206 214	256 260	237 241	164 166	124 132	174 180	149 151	132 138	168 182
	AGP95145		206 216	254 262	233 233	162 164	126 130	156 168	149 153	128 132	182 182
	AGP95153		206 206	256 260	227 237	164 166	132 140	174 174	147 151	132 132	166 168
1998	W1222/23		206 218	260 262	233 237	164 166	110 124	168 180	149 149	132 132	168 182
	AGP98002		206 218	262 262	237 245	164 166	124 140	168 174	149 151	130 132	168 182
	AGP98049		206 216	260 260	245 245	164 164	130 136	178 180	145 145	122 126	168 176
1998	W5623/24		216 216	248 254	231 233	164 164	128 130	174 180	137 147	132 136	170 172
	AGP98006		216 218	248 254	231 233	164 166	116 128	176 180	145 147	126 136	168 172
	AGP98099		216 216	— —	— —	164 164	102 130	148 176	145 153	130 136	166 168
1999	W5049/50		206 206	256 258	231 233	162 164	134 134	152 180	149 153	122 140	168 176
	AGP99044		206 206	256 260	231 231	162 164	116 134	152 168	145 153	132 140	176 182
	AGP99045		206 218	258 260	231 237	164 164	124 136	164 176	145 147	130 144	168 174
2000	W6232/33		206 216	248 256	233 237	164 166	130 132	154 178	145 149	130 138	168 176
	AGP00059		206 216	234 250	237 245	164 164	128 130	180 184	145 149	94 124	174 184
	AGP00065		216 218	248 248	225 231	166 184	110 130	154 180	147 149	136 138	166 168
2000	W6264/65	Dizygotic	206 216	250 256	233 233	164 166	116 140	148 154	149 149	130 130	176 178
	AGP00092		206 214	256 260	233 245	164 166	116 140	154 182	137 149	126 130	172 178
	AGP00093		214 216	256 260	215 233	164 166	110 116	154 182	137 149	126 130	172 178
2000	W6407/07		206 216	252 258	237 237	164 166	112 116	166 174	149 149	134 138	168 168
	AGP00125		206 218	252 262	231 231	164 166	116 126	166 174	149 149	132 134	168 174
	AGP00126		206 216	248 248	233 237	164 164	100 110	174 180	145 149	136 144	168 176
2000	W5641/42		214 218	260 266	227 233	164 164	102 114	166 178	149 151	126 136	166 168
	AGP00137		208 214	266 266	227 245	164 166	102 126	154 178	149 151	126 130	168 168
	AGP00139		206 206	256 260	233 245	164 166	128 130	148 152	147 147	126 130	168 170
2000	W5006/07	Dizygotic	206 216	260 262	233 237	164 184	102 126	156 182	147 149	128 136	168 182
	AGP00491		206 206	260 262	229 237	164 184	126 126	156 174	145 147	128 130	168 182
	AGP00492		206 206	252 262	237 237	164 166	102 126	156 168	149 149	128 136	168 182
2002	W6287/88	Monozygotic	208 216	234 260	229 241	164 164	110 126	154 166	137 149	122 132	168 182
	AGP02113		216 218	— —	215 229	164 164	126 126	160 166	137 149	122 126	166 182
	AGP02114		216 218	254 260	— —	164 164	126 126	160 166	137 149	122 126	166 182

seals breeding at McMurdo Sound in Antarctica. These authors reduced their original estimate based on behavioral observations after finding that only 3 of 7 putative pairs of twins were genuine. Similarly, genetic analysis allowed us to revise our initial estimate of the twinning rate (0.21%, 11 of 5,194 pups born during the study) downward to 0.06% (3 of 5,194). However, it is important to note that our study was not designed specifically to quantify the twinning rate and it is possible that we did not observe every instance of twinning in the colony.

Factors affecting twinning.—Twins are probably rare in pinnipeds because of the high energetic demands of milk production during a short but intensive suckling period (Doidge 1987; Haase 2007; McMahon and Hindell 2003). Consequently, it is noteworthy that all 3 of the pairs of twins were born during seasons of relatively high pup production (713 and 769 pups, respectively, in 2000 and 2002, compared with an annual average of 649 pups during the study period). Although our sample size is small, this finding is suggestive of a tendency for twin births to occur in favorable years when food is plentiful and females are in above-average condition. Further support for a possible link between condition and twinning comes from the observation that 2 of the 3 mothers of twins were not observed rearing pups during the season in which the twins were conceived (female W6264/65 was absent from the colony and female W5006/07 was present but without a pup). Such an observation could imply that individual females who skip breeding may be better able to accumulate sufficient resources to subsequently support the gestation of twin pups.

Birth weight and survivorship.—Pinniped twins are often born undersized (Spotte 1982) and tend to grow more slowly than singleton pups (Doidge 1987; McMahon and Hindell 2003). Despite this, however, female fur seals have occasionally been observed successfully weaning 2 pups (Bester and Kerley 1983; Doidge 1987; Haase 2007). Interestingly, all but 1 of the twin pups identified in this study had birth weights falling within the normal range for the colony and, with the exception of the pup that was born underweight, all survived until the end of the pupping period. Although we were unable to observe the pups up to weaning, this finding lends additional support to the notion that female fur seals are sometimes capable of simultaneously rearing more than 1 pup.

Zygotic status and paternity.—In contrast to the study of Gelatt et al. (2001), which identified 3 pairs of dizygotic twins in Weddell seals, we found 2 pairs of dizygotic twins and 1 pair of monozygotic twins. The discovery of a pair of monozygotic twins is noteworthy because these tend to be rare in pinnipeds. For example, Spotte (1982) found that only 3 of 25 documented cases of seal twins for which zygotic status could be determined were monozygotic. Moreover, to our knowledge this study is the 1st to genetically demonstrate the presence of monozygotic twins in a natural pinniped population.

Using paternal exclusion testing, paternity was successfully assigned to the monozygotic twins, but unfortunately it was not possible to identify the fathers of the dizygotic twins, precluding the most direct means of testing whether multiple paternity occurs in this species. Our inability to assign paternity is perhaps surprising given that we sampled the majority of territory-holding males present during the study ($n = 464$—Hoffman et al. 2004). As noted previously, however, neither of the mothers of the dizygotic twins were seen rearing pups during the year in which the twins were conceived. Hoffman et al. (2003) found that a female Antarctic fur seal's maternal status at conception was an important determinant of the paternity of her pups, with females that were absent from the study colony or nonpregnant being far less likely to conceive to a territorial male sampled on the study beach than those with pups. It therefore follows that both pairs of dizygotic twins were probably conceived away from the study colony. This in turn suggests that, even with more extensive sampling in the future, it may prove difficult to substantially enlarge our sample size of twins with known paternity in this species.

Despite this setback we were able to determine the relative statistical support for the competing hypotheses of twins being full versus half siblings using log-likelihood tests implemented in the program Kinship (Goodnight and Queller 1999). Although 1 of the pairs had a significantly greater likelihood of being full siblings against the null of half sibship, the other was significantly more likely to be half siblings against the null of full sibship, indicating probable multiple paternity. Taken at face value, if female fur seals are monestrous as believed by Boyd (1991), this finding suggests that they may on occasion mate with >1 male at or around estrus, and hence that sperm competition could play an unexpected role in the mating system of this species. This new insight, taken together with the recent discovery that female fur seals exert active mate choice (Hoffman et al. 2007), suggests that the reproductive biology of this species could be more complex than originally thought.

Conclusion.—Our findings, together with those of Gelatt et al. (2001), show that molecular genetic analysis can make a valuable contribution toward the study of twinning in natural pinniped populations. Following the recent publication of a number of new microsatellite loci for otariids (e.g., Hoffman et al. 2008; Huebinger et al. 2007) it should become increasingly possible to conduct similar studies using fewer but more polymorphic markers, further enhancing the appeal of this approach.

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