

ORIGINAL ARTICLE

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Mate choice among sympatric fur seals: female preference for conphenotypic males

Received: 19 January 1998 / Accepted after revision: 12 September 1998

Abstract When closely related species breed in sympatry, and where hybrids have lower fitness, reinforcement theory predicts that selection should favour mechanisms that reduce the probability of interspecific matings. If this situation arises among species that exhibit resource defence polygyny where males and females of different species reside in the same territories, there may be some conflict between mate choice based on territory-holding ability (sexual selection) and mate choice for correct species. We investigated this in a population of fur seals where three species are sympatric and where some females breed in the territories of heterospecific males, and where interspecific matings and hybrid pups are observed. The territorial status of males and the birthing sites of females were determined during daily observations, as were the movements of males and females, the location of matings and mating partners. DNA extracted from skin samples was used to determine paternities using DNA fingerprinting and the mtDNA genotype of individuals. Individuals were also classed on the basis of species-typical phenotype. We found that extra-territory inseminations (ETIs) were significantly more prevalent (67%) when territorial males and resident females were of different phenotype than when of similar phenotype (27%), but mtDNA genotype had no effect on the rate of ETIs. ETIs were probably by males with the same phenotype, as pups born to these females in the following season had the same phenotype as their mothers, suggesting they were not hybrids. These results suggest that within the resource defence polygynous

mating system of these sympatric fur seals, female mate choice is more influenced by male phenotype than genotype. Contrary to our predictions, our study indicates that potential conflict between mate choice based on sexual selection and species recognition is unlikely, because females have some capacity to discriminate between males both within and between species on phenotypic traits additional to those under sexual selection. Although at least 25% of the pups born in this study were hybrid, this study can only support reinforcement theory if hybrids have reduced fitness. The fitness of hybrids among the species studied is currently unknown.

Key words Mate choice · Mating success · Hybridization · DNA fingerprinting · Mitochondrial DNA · Fur seal · *Arctocephalus* spp.

Introduction

An assumption often made for mammals exhibiting resource defence polygyny is that females choose breeding sites based on the resources of the sites and that females are then passive recipients of matings by the males in whose territories they reside (Emlen and Oring 1977). Females not only gain the direct benefits of the resources, but they might also gain indirect benefits, through sexual selection, of high-quality males who are able to compete successfully for the resources and access to oestrous females (Andersson 1994). However, when closely related species which exhibit resource defence polygyny breed sympatrically, such that males and females of different species may reside in the same territories, there may be some conflict for females between choosing males on the basis of their territory-holding ability (sexual selection) versus their species status.

The selective benefits of species recognition have been suggested to be important in the evolution of mate choice and secondary sex traits (see Andersson 1994 for review). Where two divergent populations come into

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contact, natural selection will favour assortative mating if hybrid progeny have lower fitness (Dobzhansky 1940; Butlin 1987, 1995). This theory, termed 'reinforcement', may complete speciation when post-zygotic barriers are incomplete (Butlin 1987, 1995; Rice and Hostert 1993; Rundle and Schluter 1998). Speciation by reinforcement requires strong selection against hybrids. However, two major processes may counter it: first, recombination, which may disrupt gene combinations that promote hybrid sterility or inviability, and secondly, gene flow from other non-sympatric populations, which may undermine the selection for assortative mating (Butlin 1995).

Whereas reinforcement is the evolutionary process whereby selection promotes recognition (and hence assortative mating) among species or divergent populations breeding sympatrically, reproductive character displacement is the selection of enhanced specific differences in traits, courtship or preference in areas of sympatry relative to areas of allopatry (Brown and Wilson 1956; Howard 1993). Most empirical studies of reinforcement have focused on the presence or absence of reproductive character displacement, which has been found in both experimental and field studies (Coyne and Orr 1989, 1997; Howard 1993; Noor 1995, 1997; Albuquerque et al. 1996; Saetre et al. 1997; Rundle and Schluter 1998), although not in all (Doherty and Howard 1996).

The congeneric fur seals in the genus *Arctocephalus* represent a recently radiated group of eight species which predominate in the southern hemisphere. Several of these species have ranges which overlap with other species, including Australian (*A. pusillus doriferus*) and New Zealand fur seals (*A. forsteri*) (Goldsworthy et al. 1997), Juan Fernandez (*A. phillippii*) and subantarctic fur seals (*A. tropicalis*) (J.M. Francis, personal communication), and Antarctic (*A. gazella*) and subantarctic fur seals (Condy 1978; Kerley and Robinson 1987; Shaughnessy et al. 1988). At Macquarie Island in the south-western Pacific Ocean, three fur seal species occur in sympatry: Antarctic, subantarctic and New Zealand fur seals (Shaughnessy and Goldsworthy 1993). Hybrids between Antarctic and subantarctic fur seals have been reported from two of the three islands where these species breed sympatrically; these have been inferred from individuals with intermediate external characteristics and skull morphometrics (Condy 1978; Kerley and Robinson 1987; Shaughnessy et al. 1988). At Macquarie Island, fur seals breed in mixed species colonies where breeding territories may contain both female antarctic and subantarctic fur seals, and territorial males of either species or New Zealand fur seals. Interspecific matings have been observed and phenotypic hybrid pups also occur (Shaughnessy et al. 1988).

The unique fur seal population at Macquarie Island provides a natural experiment enabling simultaneous investigation of the influence of species recognition and male resource-holding potential on female choice and male mating success. We compared the mating success of males holding territories comprised of conspecific and

heterospecific females, and determined the discriminatory abilities of females by assessing the extent to which they obtained extra-territorial inseminations (ETIs) from DNA fingerprinting in relation to whether territorial males were of the same phenotype or mtDNA genotype. If females in territories of heterospecific males obtain more ETIs than females in territories with conspecific males, we infer this as evidence for assortative mating and species recognition.

Methods

Study site

Macquarie Island (54°S 159°E) is situated in the south-western Pacific Ocean, just north of the Antarctic Polar Front. The original fur seal population at Macquarie Island was exterminated by sealers by about 1820, and records indicate that prior to exploitation, the population numbered at least 190,000 individuals. However, the identity of the original species is unknown (Shaughnessy and Fletcher 1987). Fur seals did not breed on the island again until 1955, and since then the population has been increasing at a rate of 10.5% per annum (Shaughnessy and Goldsworthy 1993). Of the three species of fur seal that occur on the Island, Antarctic and subantarctic fur seal are the most numerous breeding species (Shaughnessy et al. 1988). The New Zealand fur seal population on the island consists of mostly non-breeding males, but males of this species occasionally hold breeding territories. The main concentration of breeding on the island occurs in two small bays (Secluded Beach and Goat Bay) on North Head Peninsula, on the northern tip of the island (Fig. 1). The study was conducted over three breeding seasons in the austral summers of 1991/1992–1993/1994. In these years, the total pup production for the island was 79, 86 and 96, respectively. This study was conducted at Secluded Beach where 86%, 90% and 91% of the pup births for the population occurred in the three seasons of the study, respectively.

Species identification

Species identification of individual seals was determined by both phenotypic and genetic differences among the species. Phenotypic differences among fur seal species include pelage colour and pattern, proportional differences in body and flipper shape, behaviour and vocalisations (Condy 1978; King 1983; Shaughnessy et al. 1988; Goldsworthy et al. 1997). The species identity of pups can also be readily determined by their phenotype, especially those of the Antarctic and subantarctic fur seals which differ markedly in the colouring and patterning of their natal pelage (Condy 1978; King 1983; Shaughnessy et al. 1988). On Macquarie Island, most individuals can be readily assigned to species based on such characteristics. The main species-typical characteristics used to assign a species phenotype to individual pups, females and males are summarised in Table 1. The characteristics of individual seals usually correlated well with the entire set of characteristics typical for that species, but some individuals shared characteristic of each species. We termed these individuals 'phenotypic hybrids', and they may be F₁ hybrids or backcrosses (Condy 1978).

We also used mtDNA sequences to identify species-specific genotypes (Fig. 2) and in some cases hybrids. We amplified extracted DNA using the polymerase chain reaction (PCR), with oligonucleotide primers homologous to the tRNAs on either side of the mtDNA control region, 5'-TTCCCGGTCTTGTAAC-3' (T-Thr) and 5'-ATTTTCAGTGTCTTGCTTT-3' (T-Phe) following Hoelzel and Green (1992) and Hoelzel et al. (1993). DNA was amplified in 50- μ l reactions containing 2 μ l of 0.1–0.5 μ g genomic DNA, 5 μ l 10 \times buffer (0.1 M Tris-HCl, pH 8.5, 0.025 M

Fig. 1 The location of the study site Secluded Beach, Macquarie Island, and the spatial arrangement of breeding territories and the species phenotype of the territorial males during the 1991/1992 and 1992/1993 breeding seasons. The numbers of breeding females in each territory and their species phenotype are also indicated (g Antarctic fur seal, t subantarctic fur seal, f New Zealand fur seal, *hyb* phenotypic hybrid)

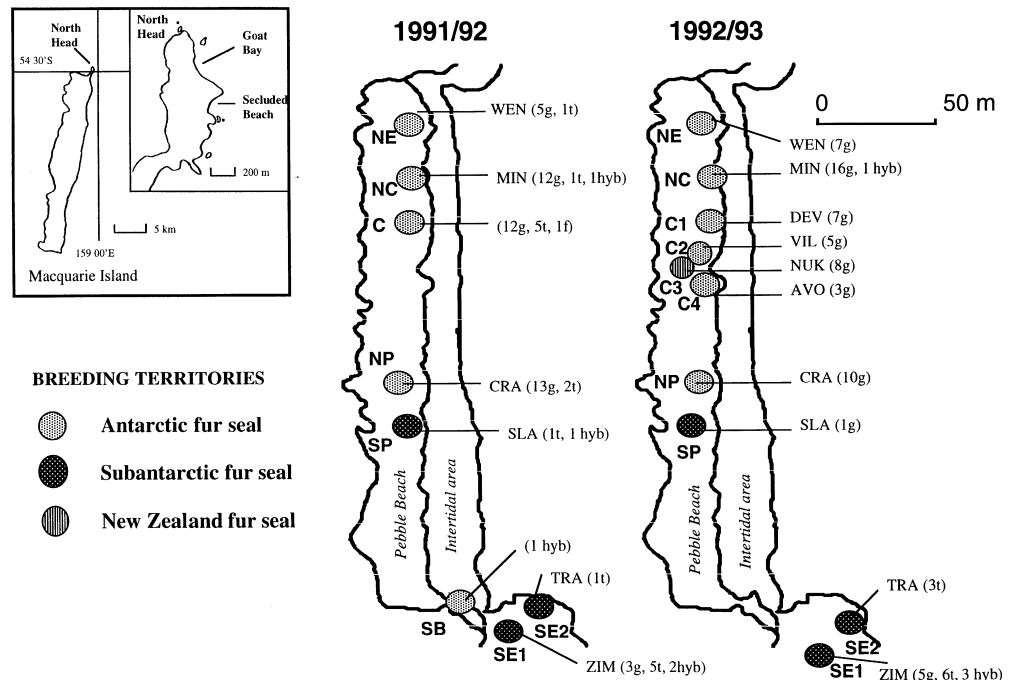


Table 1 Phenotypic characteristics typified by pups, adult male and female Antarctic, subantarctic and New Zealand fur seals. Individuals which shared characteristics of each species were termed phenotypic hybrids

Age/sex class	Antarctic fur seal ^{a,b,d}	Subantarctic fur seal ^{a,b,d}	New Zealand fur seal ^{b,c,d}
Pups (natal coat)			
Coat colour	Ash-grey	Glossy black	Dark brown
Head guard hairs	Grizzled	Black	Grizzled
Muzzle	Pale	Black	Pale
Belly	Pale	Black	Pale
Adults			
Pelage: adult females	Chest and neck cream to grey, dorsal surface grey to brown. Typically more uniform in colour than subantarctic fur seals	Pale cream to yellow bib extends from chest above the eyes but below the ears and over the bridge of the nose. Dorsal surface grey to dark brown and black	Typically uniform brown, although lighter and grey after the moult. Lighter ventrally
Pelage: adult males	Similar to females, uniformly grey with no crest	Similar to females but with prominent crest of black hair, raised when agitated	Similar to females but with pale muzzle
Ears	Long, covered with pale hair often white on tips	Short covered with black hair	Brown ears
Eye shape	Almond shaped	More rounded tear shaped	Almond shaped
Hind flippers	Long and slender	Shorter and more square	Long and slender
Pup attraction call of females	High pitched often with trilling components	Mournful wail typically without trilling	High-pitched trilling

^a Condy (1978)

^b Shaughnessy et al. (1988)

^c Goldsworthy et al. (1997)

^d Personal observations

MgCl₂, 0.5 M KCl), 5 µl dNTPs mix (2 mM dATP, dTTP, dCTP, dGTP, in 0.1 M Tris-HCl, pH 7.9), 3 µl of a 10 µM solution of each primer, 0.3 µl Taq DNA polymerase, and 26.7 µl deionised water. The cycle profile was 2 min at 94 °C, 2 min at 50 °C, and 45 s at 70 °C, repeated 30 times. Sequencing was performed using automated DNA sequence analysis employing dye-labelled terminators (Carr and Marshall 1991; Ferl et al. 1991; Applied Biosystems 1994). The PCR products were sequenced with the T-Thr primer, and an additional internal control region primer

(SCR-1, 5'-CCTGAAGTAAGAACCAGATG-3') (Hoelzel et al. 1993).

Using phenotype and mtDNA genotype data, we defined the relationship between females within a breeding territory and the territorial male as 'conphenotypic' when they shared the same species-typical characteristics, and 'heterophenotypic' when they did not. Similarly, territorial males and breeding females within the territory were defined as 'congenotypic' when they shared the same mtDNA genotype and 'heterogenotypic' when they did not.

Fig. 2 Aligned mtDNA control region sequences of 100 base pairs for Antarctic (*A. gazella*), subantarctic (*A. tropicalis*) and New Zealand (*A. forsteri*) fur seals. The 11 species-specific bases used to assign genotype are marked with an *

	0									50
									**** *	
<i>A. gazella</i>	CCAACACCCA	AAGCTGACGT	TCTAATTAAA	CTATTCCCTG	ACATATTAAT					
<i>A. tropicalis</i>	GACT.A	
<i>A. forsteri</i>	C..A	
	51									100
		*		*	*	*	*		*	
<i>A. gazella</i>	CTCCCCATAT	TCATATGTAT	CACTACACCC	ACTGTGCCAT	CATAGTATCT					
<i>A. tropicalis</i>C..A..C	...C.....C	
<i>A. forsteri</i>A...T..	

Pup paternity

We isolated DNA from skin biopsy samples, stored in a salt-saturated solution of 20% DMSO, obtained from 38 females for which the territory where they gave birth and resided during the peri-natal period was known for the previous breeding season. For 15 females this was known for two breeding seasons, giving a total of 53 mother-pup pairs for the study. We considered each female-pup pair as an independent event. All territorial and as many challenger males as possible (10 and 10, respectively over the two seasons) in the population were sampled. Biopsy samples were obtained from the rump of adult females and males using a 6-mm-diameter biopsy punch with a barb cut into it, fixed to the end of a long pole. Samples were obtained from pups born in the 1992/1993 and 1993/1994 breeding seasons, by pushing a biopsy punch through the trailing edge of the fore-flipper prior to tagging. These samples provided data on inseminations from the 1991/1992 and 1992/1993 breeding seasons, respectively. DNA was isolated in the laboratory using standard proteinase K digestion, phenol-chloroform extraction and ethanol precipitation. DNA fingerprinting followed the protocol of Loew and Fleischer (1996). Membranes were hybridised with ³²P-labelled minisatellite probes, Jeffrey's 33.15 (at 58 °C) and 33.6 (at 55 °C, for a subset of gels). Membranes were washed in 2 × SSC:0.1%SDS at the above temperatures and then exposed on autoradiographs for up to 14 days. The DNA of mother-pup pairs, including DNA from the putative father (territorial male) and males from adjacent territories was run on the gels in order to determine the paternity of pups, and hence which males had successfully inseminated females. The average number of fragments measured per lane using both probes was 14.6 (SD = 4.5, n = 274). Paternity was ascribed when the similarity coefficient (S) between the male and the pup was in the range of that found for mother-pup pairs (mean = 0.64, SD = 0.10, n = 53), and the male had all, or all but one, of the non-maternal bands in the pup's profile. S for unexcluded males was 0.59 (SD = 0.11, n = 40). S for mother-pup and father-pup pairs did not differ significantly from the expected S for first-degree relatives of 0.61 (Lynch 1991) (t = 0.041, df = 52, P > 0.05; t = 0.287, df = 39, P > 0.05, respectively), given that a sample of unrelated individuals had an S of 0.28 (SD = 0.09, n = 37 independent samples). Both the S of mother-pup and father-pup pairs differed significantly from those of unrelated individuals (t = 16.9, df = 87, P < 0.001; t = 13.3, df = 75, P < 0.001, respectively).

Male mating success

Typically, measures for estimating individual male mating success in pinnipeds include (1) the numbers of females associated with a male, (2) the number of pups born in close proximity to a male and (3) the number of copulations achieved by a male (Boness et al. 1993). Using genetic techniques to determine paternity in pinnipeds is difficult because young from successful matings are born 1 year later and pregnant females may not always return to where they mated, or produce a pup in the following season. We were able to determine paternities through multilocus DNA fingerprinting, and estimate two measures of mating success (MS), defined as

$$MS = [(F_t/F_{t_s}) \times F_t] + F_{x_t}$$

where F_{t_i} is the number of sampled females in a male's territory inseminated by a male, F_{t_s} is the number of sampled females that gave birth in a male's territory, F_t is the total number of females in a male's territory, and F_{x_t} is the number of ETIs obtained by a male. Note that inseminated females include those that produced a pup in the following breeding season and do not include those that may have aborted their pup prior to the pupping season. The second measure was relative rating success (RMS), which was defined as

$$RMS = MS/F_p$$

where F_p is the total number of females which pupped in the study population. RMS was only calculated for the 1992/1993 season, when all territorial males were sampled, and the identity of successful inseminators was determined for nearly half the females (49%, 37/76).

Behaviour of males and females during the peri-natal period

The peri-natal attendance period in otariid seals (fur seals and sea lions) includes the period from when females haul-out to give birth (usually 1–2 days prior to parturition) and the period after this when mothers are in continuous attendance of their pup (usually 7–10 days post-partum). Females usually come into oestrus and are mated about 7 days post-partum (Boness 1991). The behaviour of females during the peri-natal attendance period was monitored over a total of 59 h between 3–19 December 1992. The maximum number of hours observed on any given day was 4. Observations were recorded on three types of behaviour: (1) the number of interactions between territorial males and females within the territory, (2) which sex initiated interactions and (3) attempts made by females to leave the male's territory. These data were examined in relation to the number of days since parturition and proximity to oestrus for each female, and to whether the territorial male and female were conspecific or congenotypic. Observations were undertaken from the periphery of breeding territories with the aid of binoculars.

Territorial males were defined as males defending territories containing breeding females, while challenger or non-territorial males were defined as males in loosely defined territories without breeding females. Territory boundaries were inferred from daily observations of male behaviour. Individual females and males were recognised by tags, bleach marks or by scars. Daily observations were made to determine the location of individual seals and record the parturition date, location and presence of females. The mean timing of oestrus (days post-partum until mating) was determined for 29 different females seen to mate over four breeding seasons (1990/1991–1993/1994).

Results

Spatial arrangement and species composition of breeding territories

The number of males of each species (based on phenotype) that held territories during the 1991/1992 and

1992/1993 breeding season at Macquarie Island, and the number of females that gave birth in them is presented in Fig. 1 and Table 2. There were 11 (8 in Secluded Beach) breeding territories in which pups were born in 1991/1992, and 13 (10 in Secluded Beach) in 1992/1993 (Fig. 1). The number of territories held by Antarctic and subantarctic males at Secluded Beach was about the same in each year (Table 2). One territorial male with a New Zealand fur seal phenotype held a breeding territory in the 1992/1993 season (Table 2, Fig. 1). The mean number of females that gave birth in the territories of Antarctic fur seal males was significantly greater than the number that pupped in those held by subantarctic fur seal males in 1991/1992 (Antarctic fur seal: mean = 9.5, SD = 5.2, range 1–15, $n = 6$; subantarctic fur seal: mean = 3.0, SD = 3.9, range 1–10, $n = 5$; $t = 2.27$, $df = 9$, $P = 0.049$), but not in 1992/1993 (Antarctic fur seal: mean = 8.2, SD = 4.9, range 3–17, $n = 6$; subantarctic fur seal: mean = 3.6, SD = 4.8, range 1–14, $n = 5$; $t = 1.70$, $df = 11$, $P = 0.118$).

The spatial arrangement of breeding territories in the population was such that few had common boundaries (Fig. 1). With the exception of the territories C2, C3 and C4 (in 1992/1993), most breeding territories were disjunct with at least 10 m separating their boundaries (Fig. 1). In both breeding seasons, the majority of

Antarctic fur seal females gave birth in territories held by conphenotypic males (Table 3). However, more Antarctic fur seal females gave birth in territories held by heterophenotypic males (subantarctic and New Zealand fur seals) in the second season than in the first (26% and 12%, respectively) (Table 3). The pattern was reversed for subantarctic fur seal females, 63% of which pupped in territories held by heterophenotypic males in 1991/1992, while in 1992/1993 all subantarctic fur seal females pupped in territories held by conphenotypic males (Table 3). All females which gave birth in each season did so within the boundaries of a male's territory.

Although there were similar numbers of Antarctic and subantarctic fur seal males holding breeding territories in 1991/1992 and 1992/1993, Antarctic fur seal females (based on phenotype) accounted for the majority of breeding females in each year of the study (68% and 79%, respectively), while subantarctic females accounted for only 26% and 15% of the breeding females in each year, respectively (Table 2). About 5% of females in each year had mixed phenotypes and may have been hybrids or backcrosses. Over the two breeding seasons, 51% and 70% of the pups born had Antarctic fur seal phenotypes, while 28% and 23% had subantarctic fur seal phenotypes, respectively (Table 2). The

Table 2 The number and percentage (in parentheses) of territorial males, breeding females and pups of each species based on phenotype in the entire fur seal population at Macquarie Island (*Total*)

		Location	Antarctic fur seal	Subantarctic fur seal	New Zealand fur seal	Mixed phenotype	No record
1991/1992							
Territory males	Total		6 (55)	5 (45)			
	SB		5 (62)	3 (38)			
Adult female	Total		50 (68)	19 (26)	1 (1)	4 (5)	
	SB		47 (75)	12 (19)		4 (6)	
Pups	Total		39 (51)	21 (28)		16 (21)	3
	SB		37 (57)	14 (21.5)		14 (21.5)	3
1992/1993							
Territory males	Total		6 (46)	7 (46)	1 (8)		
	SB		6 (60)	3 (30)	1 (10)		
Adult female	Total		64 (79)	12 (15)	1 (1)	4 (5)	
	SB		61 (82.4)	9 (12.2)		4 (5.4)	
Pups	Total		57 (70)	19 (23)		5 (6)	4
	SB		57 (75)	15 (20)		4 (5)	1

and for Secluded Beach (*SB*) during the breeding seasons of 1991/1992 and 1992/1993

Table 3 The number and percentage of adult females of each species (based on phenotype) which gave birth in the breeding territories held by males of each species in the 1991/1992 and 1992/1993 breeding season, for the entire fur seal population at Macquarie Island

Adult females	1991/1992 territorial males			1992/1993 territorial males		
	Antarctic fur seal	Subantarctic fur seal	New Zealand fur seal	Antarctic fur seal	Subantarctic fur seal	New Zealand fur seal
Antarctic fur seal	44 (88%)	6 (12%)	–	48 (74%)	9 (14%)	8 (12%)
Subantarctic fur seal	12 (63%)	7 (37%)	–	–	12 (100%)	–
New Zealand fur seal	1 (100%)	–	–	–	–	1 (100%)
Hybrid?	2 (50%)	2 (50%)	–	1 (25%)	3 (75%)	–

percentage of phenotypic hybrid pups declined from 21% to 6% between 1991/1992 and 1992/1993, respectively (Table 2). The spatial separation of most of the Antarctic fur seal females (concentrated on the cobble beach) from the subantarctic females (concentrated on the rocky section of the southern end of Secluded Beach) was clear (Fig. 1), and may indicate species-specific habitat preferences. Such differences in habitat preference between these species has been noted previously (Condy 1978; Wilkinson and Bester 1990).

Territory and extra-territory inseminations

Over the two seasons, the paternity of the territorial male could be confirmed or excluded for 53 female-pup pairs (38 individual female-pup pairs). Sixty-six percent (35) of these gave birth to pups sired by the territorial male of the preceding season, while 34% (18) produced pups sired by an extra-territory male (ETM) (Table 4). Males from an adjacent territory were sires in 6 (33%) of the 18 ETIs. In all other cases (66%), ETMs were un-

Table 4 The phenotype and mtDNA genotype of territorial males and females which gave birth in their territories (*G* Antarctic, *T* subantarctic, *F* New Zealand fur seal, *Mixed* phenotypic hybrid). Individuals with a phenotype inconsistent with their mtDNA genotype are indicated with an *asterisk*. Results from DNA fingerprinting, indicating whether females were inseminated by their

territorial male (*TI*) or an extra-territorial male (*ETI*), are also given. The phenotype of the resultant pup born in the season following insemination is listed. Putative hybrid or backcrossed pups based on phenotype and mtDNA genotype are denoted with the superscripts *PH* and *MH*, respectively

Territorial male	Female	Phenotype	mtDNA genotype	Fertilisation 1991/1992	1992/1993 pup phenotype	Fertilisation 1992/1993	1993/1994 pup phenotype
ZIM		T	T				
	594	G	G	ETI	G	ETI	G
	602	Mixed	G*	TI	Mixed ^{PH, MH}	TI	Mixed ^{PH, MH}
	560	Mixed	G*	TI	T ^{MH}	TI	Stillborn ^{MH}
	A06	G	G	TI	G ^{MH}	ETI	G
	604	T	G*	TI	T ^{MH}	TI	T ^{MH}
	509	T	T	TI	T	TI	T
	521	T	T	TI	T	TI	T
	0018	T	G*	TI	T ^{MH}	TI	T ^{MH}
	990	T	G*	TI	T ^{MH}	TI	T ^{MH}
SLA		T	T				
	A01	G	G	TI	T ^{PH, MH}	TI	G ^{MH}
NUK		F	G*				
	421	G	G			ETI	F
	416	G	G			ETI	G
	0093	G	G			ETI	G
MIN		G	G				
	579	G	G	ETI	G	TI	G
	546	Mixed	G*	ETI	G	ETI	Mixed ^{PH}
	568	G	G			ETI	G
	952	G	G			ETI	G
	977	G	G			ETI	G
	979	G	G			TI	G
	966	G	G			TI	G
	969	G	G			TI	G
CRA		G	G				
	501	G	G	TI	G	TI	G
	0095	G	G	TI	G		
	397	G	G	TI	G	TI	G
	588	G	G	TI	G	TI	G
	0060	G	G	ETI	G		
	998	G	G			TI	G
DEV		G	G				
	390	G	G			TI	G
	570	G	G			TI	G
	970	G	G			TI	G
	950	G	G			ETI	G
VIL		G	G				
	964	G	G			TI	G
	0060	G	G			ETI	G
	0007	G	G			ETI	G
WEN		G	G				
	614	G	G			ETI	G
	A07	G	G			TI	G
	973	G	G			ETI	G
	A00	G	G			TI	G

identified, but DNA fingerprinting analyses excluded other territory holders and the challenger males sampled as possible fathers, suggesting that most ETIs resulted from matings with unsampled challenger and non-territorial males. These males were not completely sampled because they either moved frequently or were present for only a short period of time during the breeding season.

Among conspecific territories where both the territorial male and females expressed an Antarctic fur seal phenotype and mtDNA genotype, the prevalence of ETIs was similar to that above (36%, $n = 28$, data from both years; 32%, $n = 22$ data from 1992 breeding season, Table 4). Comparisons with subantarctic fur seals are not possible, due to small sample sizes (Table 4).

ETIs were relatively infrequent (26%, $n = 38$ excluding females that were phenotypic hybrids) when females were within territories held by a conphenotypic male, but were prevalent when the territorial male was heterophenotypic (67%, $n = 9$ excluding females that were phenotypic hybrids) (Table 4). The difference between the frequency of ETIs in territories held by conphenotypic and heterophenotypic males was significant ($G\text{-adj} = 4.71$, $P < 0.05$). Males that obtained the ETIs were likely to be conphenotypic to the females because the phenotypes of all pups born from such matings in the following season were the same as those of their mother (Table 4). All three of the phenotypic hybrid females (Table 4) had Antarctic fur seal mtDNA genotypes, suggesting that their mothers or maternal grandmothers were Antarctic fur seals (or hybrid) while their fathers were likely to have been subantarctic fur seals.

Contrary to the results based on phenotype, an examination of the level of ETIs relative to the mtDNA genotypes of the territorial male (Table 4) showed no significant difference in the level of ETIs among females in territories held by congenotypic males (41%, 15 of 37) and those held by heterogenotypic males (19%, 3 of 16) ($G\text{-adj} = 1.23$, $P > 0.10$).

Male mating success

The mean mating success of territorial males in the 1992/1993 breeding season, irrespective of the species identity of females was 5.6 ($SD = 4.43$, range 0.0–10.9, $n = 8$) (Table 5). Relative mating success ranged from 0 to 14.3%, with a mean of 6.1% ($SD = 5.7$, $n = 10$) (Table 5). When heterophenotypic females were excluded from the analysis, the average mating success of territorial males that mated increased to 6.6 ($SD = 3.35$, range: 1.0–10.0, $n = 6$). Among conphenotypics from both breeding seasons, there was a significant relationship between the number of females in a male's territory and his mating success (linear regression, $t = 2.73$, $df = 7$, $P = 0.034$, $r^2 = 0.554$; cases where <20% of females in a territory were sampled was excluded). Overall, territorial males inseminated 66% (41) of the 53 females that gave birth in their territories, sampled over

the two breeding seasons. An additional 11% were inseminated by territorial males from neighbouring territories, and the remaining 23% (12) by challenger males or other non-sampled males.

Copulations did not assure paternity; in two of five observed copulations, females did not bear the observed male's pup in the following season. The three successful copulations were between conphenotypes (Antarctic fur seal), while the two unsuccessful copulations were between heterophenotypes (subantarctic male/Antarctic female; New Zealand male/Antarctic female). Territorial males had a significantly higher success inseminating conphenotypic females in their territories (60% inseminated) compared with heterphenotypic females (7% inseminated) (1992/1993 data: $G\text{-adj} = 6.49$, $P < 0.020$, Table 5). The percentage of conphenotypic females (60%) in a male's territory inseminated did not differ significantly from that of hybrid females (75%) (1992/1993 data: $G\text{-adj} = 0.076$, $P > 0.70$, Table 5).

Behaviour of males and females during the peri-natal period

Females attempted to move from a male's territory at any time prior to oestrus (mean = 7.1 days post-partum, $SD = 1.6$, $n = 29$), although most movement occurred shortly after oestrus and was probably associated with a female's first feeding trip to sea following the birth of her pup (Fig. 3a,b). Regardless of when a female attempted to leave a male's territory, the male tried to prevent her. In this study, the duration from parturition to first absence from the male's territory was significantly shorter in females that obtained ETIs (ETI, mean = 6.6 days, $SD = 1.9$, $n = 18$) than in those that did not (mean = 8.2 days, $SD = 2.0$, $n = 28$) ($t = 2.68$, $df = 44$, $P = 0.01$) (Fig. 3c). The mean time to first absence for females which obtained ETIs corresponded to a peak in observed attempts by females to leave the territory (Fig. 3b). Hence, leaving a male's territory prior to oestrus enhanced a female's chance of obtaining an ETI (Fig. 3c).

Extent of hybridization/backcrossing

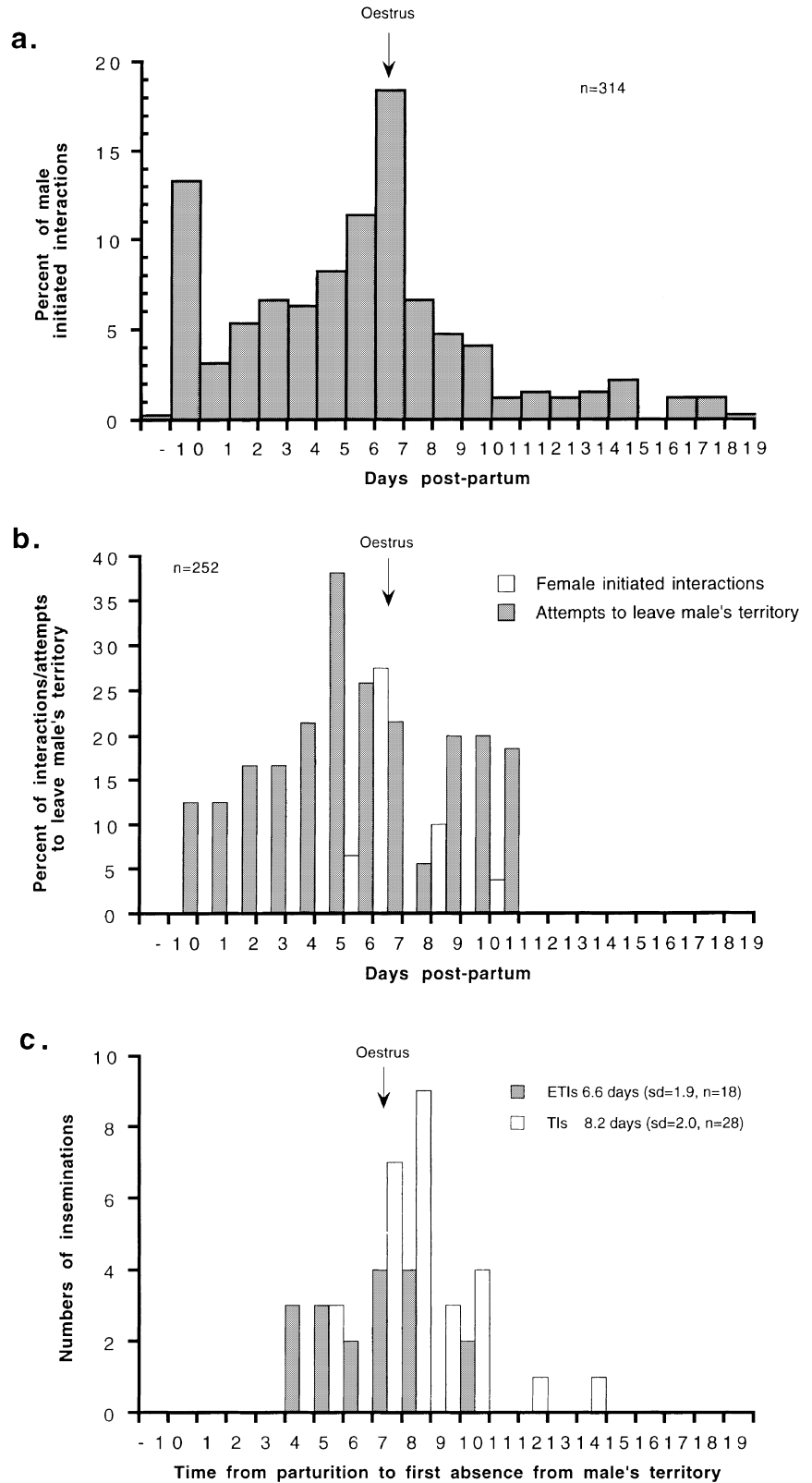
The mtDNA species-specific genotypes used in this study (Fig. 2) are maternally inherited and therefore may only indicate hybrid origin if an individual has a mtDNA genotype that differs from its phenotype. However, if an individual has a phenotype that matches its mtDNA genotype, this does not preclude it from being a hybrid through its father's lineage. Examination of these data indicate inconsistencies between the phenotype and mtDNA genotype determined for some individuals, suggesting they are hybrid (Table 4). These include three females with subantarctic fur seal phenotypes having Antarctic fur seal mtDNA genotypes, three mixed-phenotype

Table 5 The estimated mating success and relative mating success (1992 season only) of territorial males over the two seasons of the study. The mating success of males is also presented relative to the number of conphenotypic (*Con*), heterophenotypic (*Hetero*) and hybrid females within their territory. Male species identity is based on their phenotype (*T* subantarctic, *G* Antarctic, *F* New Zealand fur seal)

Male	Species	Year	Species relatedness to females in territory	Number of females in territory (a)	Females sampled which pupped in the following year (b)	Territorial females inseminated (c)	Extra-territory females inseminated (d)	Estimated mating success $[(c/b) \times a] + d = e$	Relative mating success $e/\text{sum}(a)^a$
ZIM	T	1991	All	10	9	8	0	8.9	
			Con	5	5	5		5.0	
	1992	Hetero	3	2	1		1.5		
		Hybrids?	2	2	2		2.0		
		All	14	9	7	0	10.9		0.143
		Con	6	5	5		6.0		
SLA	T	1991	Hetero	5	2	0		0.0	
			Hybrids?	3	2	2		3.0	
	1992	All	2	1	1	0	2.0		
		Con	1	0	-		-		
		Hetero	1	1	1		1.0		
		Hetero	1	1	1	0	1.0		0.013
NUK	F	1992	All Hetero	8	3	0		0.0	0.000
			All	14	2	0		0.0	
MIN	G	1991	All	12	1	0		0.0	
			Con	1	0	-		-	
	1992	Hetero	1	1	1		1.0		
		Hetero	1	1	1		1.0		
		All	17	8	4	0	8.5		0.112
		Con	16	7	4		9.1		
CRA	G	1991	Hybrids?	1	1	0		0.0	
			All	15	5	4	0	12.0	
	1992	Con	13	5	4		10.4		
		Hetero	2	0			-		
		All Con	10	6	6	0	10.0		0.132
		All Con	7	4	3	3	8.3		0.109
DEV	G	1992	All Con	5	4	0	1	1.0	0.13
			All Con	7	3	2	0	4.7	
VIL	G	1992	All Con	4	0	-		1.0	0.013
			All Con	4	0	-		1.0	
WEN	G	1992	All Con	4	0	-		1.0	0.013
			All Con	3	0	-		1.0	
AVO	G	1992	All Con	4	0	-		1.0	0.013
			All Con	3	0	-		1.0	
TRA	T	1992	All Con	3	0	-		1.0	0.013

^a Sum of females which pupped on study beach in 1992, i.e. sum column a is 76

Fig. 3 a Percentage of territorial-male-initiated interactions with females residing in their territory in relation to days post-partum. Most interactions initiated by males occurred when females gave birth and came into oestrus (7 days post-partum). **b** Percentage of female-initiated interactions and female attempts to leave the territory in relation to days post-partum. Note that female-initiated interactions with territorial males only occurred around the period of oestrus, while most female attempts to leave occurred 1 day earlier. **c** Occurrence of territorial inseminations (TIs) and extra-territorial inseminations (ETIs) (determined by DNA fingerprinting) in relation to the timing of a female's first absence from the male's territory. Note that females that depart the territory prior to 7 days post-partum, have a higher rate of ETIs (73%) than those which depart on day 7 or later (29%)



females with an Antarctic fur seal mtDNA genotype, and one male with a New Zealand fur seal phenotype and an Antarctic fur seal mtDNA genotype (Table 4). The best measure of levels of hybridization comes from pups whose paternity could be determined and

the mtDNA genotypes of each parent compared. On this basis, 33% (13 of 39 cases where paternity was determined) of the pups examined in this study were the product of subantarctic male/Antarctic female parents, indicating high levels of hybridization and/or

backcrossing in this population (Table 4). This figure is somewhat inflated because it does not include most pups born from ETIs. However, as indicated above, the phenotypes of these pups matched that of the mother suggesting that they were not hybrids. Therefore a closer approximation of the level of hybridization may be 25% (13 of 53 cases). An additional bias in these estimates results from the small sample size, as the number of pups used in this study accounted for only 22% of all the pups born in the 1992/1993 and 1993/1994 breeding seasons. Only 3 (23%) of the 13 known cases of hybridization/backcrossing were also classed as phenotypic hybrids on the basis of external characteristics, while 3 (75%) of the 4 phenotypic hybrids had parents of differing mtDNA genotype (Table 4).

Discussion

Hybridization

Although instances of interspecific mating have often been reported among pinniped species (Wilson 1975; DeLong 1982; Stewart et al. 1987; Boness et al. 1993; Miller and DeLong 1996), records of hybridization are comparatively rare. Most have been among the otariid seals, between northern fur seals and either California sea lions (*Zalophus californianus*) or the Guadalupe fur seal (*A. townsendi*) (R. L. DeLong, personal communication); presumed hybrids between Juan Fernandez and subantarctic fur seals (Francis in Boness et al. 1993); and many inferred accounts between Antarctic and subantarctic fur seals (Condy 1978; Kerley 1983; Kerley and Robinson 1987; Shaughnessy and Fletcher 1987; Shaughnessy et al. 1988). The only account in pinnipeds that has been substantiated using molecular techniques is a hybrid between a harp pagophilus groenlandicus and hooded (*Cystophora cristata*) seal (Kovacs et al. 1997). Using molecular genetic techniques, this study has detected high levels of hybridization (up to 25%) between Antarctic and subantarctic fur seals breeding at Macquarie Island. All of the hybrid pups identified using the mtDNA genotypes of their parents were the result of matings between Antarctic fur seal females (or hybrids with an Antarctic fur seal maternal lineage) and subantarctic fur seal males. No hybrids with subantarctic mothers and Antarctic fur seal fathers were reported. In the majority of these cases, hybrid pups were not recognised as phenotypic hybrids based on external features.

It is not known whether hybrids between the three species of fur seal which occur on Macquarie Island have significantly reduced fitness compared with non-hybrids. Certainly among females, there are several examples in this study of putative hybrids producing pups. One territorial male was also identified as a putative hybrid, and although this male was observed to copulate with two

females, he was unsuccessful in fertilising any females in the population. We do not yet know if this lack of success was due to any post-mating isolating mechanisms, such as hybrid inviability or zygotic mortality, or whether hybrid male fur seals are uniformly sterile as predicted by Haldane's law, where the heterogametic sex is usually sterile (Wu and Davis 1993; Wu et al. 1996). A long-term demographic study has commenced in this population to investigate, among other things, if there is differential fitness of hybrids and non-hybrids.

One potential major barrier to hybridisation between Antarctic, subantarctic and New Zealand fur seals is the substantial difference in the length of lactation in these species. Lactation in Antarctic fur seals lasts about 4 months while that of subantarctic and New Zealand fur seals is about 2.5 times longer, around 10 months (Kerley 1983; Doidge et al. 1986; Bester 1987; Goldsworthy 1992). If lactation length is largely under maternal control, and genes governing the growth and development of pups are bi-parentally inherited, then hybrid pups with an Antarctic fur seal mother may have their expected weaning age cut short, and consequently suffer low survival. However, if pups have some control over weaning age then such barriers to hybridisation may be insignificant. The greater energy expenditure by the mother as a consequence of an extended lactation period may incur a significant reproductive cost. Although data are equivocal regarding the reduced fitness of hybrids, studies of hybridisation among other mammals support this notion, especially with respect to sterility of hybrid males (Wu et al. 1996). Development of nuclear microsatellite markers in conjunction with mtDNA markers will help determine the species and hybrid status of individual seals. This along with long-term data on the survival and reproductive success of individuals will help establish whether there are fitness costs to hybridisation among these species.

Mating success

This is the first published study on otariid seals that has used molecular genetic techniques to estimate male mating success. The values of mating success, estimated for all territorial males in this study (species combined) and within Antarctic fur seals were highly variable but within the range of mean mating success determined by other methods reported in other studies on Antarctic (McCann 1980: 11.2; Boyd 1989: 9.2, respectively), subantarctic (Bester 1982: 6.6) and New Zealand fur seals (Miller 1975: 5.3, Troy 1997: 3.8–4.9, respectively). Estimates of male mating success in these studies have been derived either from numbers of observed copulations, or the numbers of females or pups born in a male's territory. Such estimates are at risk of errors that may differ within and among species (Boness et al. 1993). Estimates based on association may only be valid in species where females are sedentary during the peri-

natal attendance period (Gentry 1998), and are likely to be highly inaccurate in species where females make daily thermoregulatory movements (DeLong 1982; Boness et al. 1993), and in species where non-territorial males may attempt to sneak or steal mates (Boness et al. 1993). Although this study confirmed that territorial males inseminate the majority of females within their territories (66%), 11% were inseminated by neighbouring territorial males, and the remaining 23% by males not holding breeding territories at all (challengers and other males). Clearly, even in species where females are considered to be highly sedentary during the period of sexual receptivity, such as Antarctic fur seals (McCann 1980; Boyd 1989), measures of mating success based on female association may be substantially inaccurate. Similar results have been found for grey seals (*Halichoerus grypus*) and red deer (*Cervus elephus*) where behavioural estimates of mating success, although correlated with actual mating success determined by DNA fingerprinting, have been found to be inaccurate (Pemberton et al. 1992; Amos et al. 1993). In red deer, molecular studies actually showed that behavioural methods underestimated the actual success of successful males, and overestimated the success of many others who failed to sire any calves (Pemberton et al. 1992).

In this study, males had significantly greater relative success inseminating conspecific females (60%) than heterospecific females (7%) in their territories. Whether males had low success with heterospecific females because females avoided such matings, or because these copulations were unsuccessful due to sperm-egg incompatibilities or other post-mating isolating mechanisms is unknown. However, 13 of the pups born in this study had parents with different mtDNA genotypes, clearly indicating that many of the interspecific matings that occur at Macquarie Island result in successful insemination. Thus, the above results give some evidence for female promiscuity and perhaps sperm competition. This notion is supported by many studies that have reported multiple matings in female otariids (see review by Boness et al. 1993).

Although this study used paternity testing to confirm mating success, there are several potential sources of error associated with our estimates. Firstly, they do not include females without pups, primiparae females mating for the first time, or females which either failed to conceive or aborted their pups prior to the breeding season. Secondly, not all females which gave birth in a male's territory were sampled; ideally all should be, as should those from neighbouring territories. Thirdly, our estimates were restricted to territorial males, which accounted for about three-quarters of the inseminations. We have no estimates of the mating success of challenger and other males which accounted for nearly one-quarter of all inseminations. This study confirms that males with the greatest numbers of females in their territory have more mating opportunities and are likely, therefore, to have higher mating success than males with few females or none. However, the mating opportunities of territo-

rial males are not necessarily limited by the number of females in their territory, as demonstrated by four of the ten males in this study, which successfully inseminated females from adjacent territories. Clearly, alternate strategies exist for both territorial and non-territorial males to maximise their mating opportunities. That nearly one-quarter of the inseminations in this study were from unsampled non-territorial males indicates the importance of these alternate strategies and that territoriality is not an essential prerequisite for mating opportunities.

Mate choice

We investigated the possible influence of species recognition (based on male phenotype and mtDNA genotype) and sexual selection (as measured by resource-holding ability, i.e. territoriality) on female mate choice in the resource defence polygynous mating system of three sympatric fur seals. Using rates of ETIs as a means to assess female choice, our results suggest that female fur seals primarily choose to mate with males based on the male's phenotype, although the male's resource-holding ability also influences a female's choice: most inseminations are by territorial males. Females do not appear to select males on the basis of their mtDNA genotypes, as the rate of ETIs was not influenced by mtDNA genotype.

The phenotype of individuals was classified using an array of traits, including proportional differences in body and flipper shape, pelage coloration and pattern, vocalisations and behaviour. Because we were not able to conduct more detailed behavioural studies, we cannot say if there were particular components of the array of traits to which females were responding. There is ample evidence from other studies of females choosing conspecific males on the basis of one or more of these types of characteristics, and it has been suggested that female choice also favours conspicuous species-specific traits that make it easier for them to choose the correct species, especially where related species breed in sympatry (Andersson 1994). ETI rates, however, were not affected by the information conveyed in a male's mtDNA genotype, suggesting that mtDNA genotype, and perhaps other hidden genetically based traits, are not as critical to female choice as are variables of outward appearance.

An alternative, but perhaps less parsimonious, interpretation of our results depends on the notion that insemination probability can be reduced in heterospecific matings through sperm-egg incompatibility or other mechanisms. This alternative would require a "background" level of extra-territory copulation, which does appear to exist (e.g. Table 4), and that sperm from conspecific males can in some way outcompete sperm from heterospecific males, even if the heterospecific males contribute more sperm via increased copulation with the female. This would lead to an apparent reduced level of ETI in heterospecific pairings that is not a

result of female choice. Anecdotal observations (noted above) of two heterospecific copulations that did not result in insemination versus three conspecific ones that did support this possibility of such reduced fertility. Supporting female choice, however, is the observation that females leave territories of heterophenotypic males earlier post-partum than they leave those of conspecific males (Fig. 3). The potential influence of sperm competition cannot be discounted, as the failure of the heterospecific mating attempts may have resulted from sperm competition from conspecific males. Although we did not record multiple matings in this study, that the recipients of the heterospecific matings were inseminated by other males indicates that at least in these two cases, the females mated at least twice. The importance of multiple matings and sperm competition to female mate choice in fur seals is unknown; however, it is worth noting that in other otariid seals, the number of females which mate more than once ranges from 0 to 30% (see Boness et al. 1993 for review).

In most studies of mammals where resources necessary for females to breed are defensible, traits that enhance a male's ability to acquire and hold resources are selected through sexual selection because males with greater resource-holding ability obtain a greater proportion of inseminations (Emlen and Oring 1977; Andersson 1994). Although our study has shown that male mating success is correlated with the number of females in his territory, our results also show that even among conspecifics, more than 30% of females within a male's territory will mate and be inseminated by an ETM. These data indicate that although sexual selection for obtaining a territory encompassing the resources sought by breeding females is important in terms of mate choice, this is not the only trait used by females to select mates. In fact, many of the phenotypic traits that females may be using to choose males, such as pelage, shape and vocalisations, are unlikely to be ones that will also enhance a male's ability to obtain a territory, such as body size, fighting ability and endurance. Apart from the benefits of discriminating among potential mates on the basis of correct species or better breeding territory, mate choice in fur seals may be advantageous because it leads to mating with a male with (1) better fertilisation ability, (2) that better complements the partner or (3) produces offspring with higher heritable viability or other qualities (Andersson 1994). The importance of these features on mate choice of female fur seals, and what phenotypic features are used by them to discriminate among males is unknown. Nevertheless, our results suggest that selection of birthing sites by females may be independent of the quality of the male holding the territory, and that it is only after females give birth and are approaching the period of oestrus that the quality of the territorial male and opportunities for extra-territory mating may be assessed.

Given that there are several closely related fur seal species breeding at Macquarie Island, it appears that the costs of interbreeding outweigh the potential benefits

that may be gained by breeding with a male that has demonstrated territorial prowess, such that females seek to mate away from their birthing territory when it is controlled by a heterophenotypic male. Mate choice based on phenotype is likely to be an important means of species recognition, which may reduce the levels of introgression at sites where fur seal species breed sympatrically. At Macquarie Island, all the females that obtained ETIs were Antarctic fur seals. This correlated well with the occurrence of hybridisation, in that all hybrid pups identified were a result of matings between Antarctic females and subantarctic fur seal males. If females are attempting to avoid interspecific mating and the resultant hybrid offspring, then ETIs would be expected to occur more in the species with the higher prevalence of hybridisation.

Although this study did not investigate the traits females use to choose mates both within and between species, it did investigate the possible mechanisms by which females gain extra-territory mates. The main mechanism by which females exercised some choice was by escaping the territory in which they had pupped, just prior to their oestrus. Seventy-three percent of females which achieved this prior to 7 days post-partum (the average time of oestrus) obtained ETIs, while those that remained until 7 days or later had a much lower chance (29%) of obtaining an ETI. Unlike female fur seals breeding on cool subantarctic islands which remain in their breeding territories throughout most of the perinatal period, those breeding in hot climates such as populations of South American fur seals (*A. australis*) in Peru are forced to make daily thermoregulatory movements between their birth sites and the intertidal zone (Majluf 1987). Clearly the opportunity for female mate choice is much greater in this population, and recent studies have indicated that the majority of females mate away from their parturition sites (P.J. Majluf, personal communication). Mate choice based on partner fidelity is thought to operate in polygynous grey seals (*H. grypus*), where females appear to mate preferentially with males they mated with in previous seasons (Amos et al. 1995). All these studies on species whose mating system has been classified as resource defence polygyny indicate that although sexual selection on males to acquire territories which encompass female resources is an important component of mate determination, it is not the only one. Clearly, females are not passive recipients of matings from territorial males, and can exercise some choice. Although this study has shown that females can discriminate and prefer mating with the same species, the reasons for choice among individuals of the same species remain unclear, and deserve further research.

Reinforcement

Although this study provides evidence for mate choice based on species recognition as predicted by the theory of reinforcement, reinforcement can only be considered

as the cause of speciation if certain criteria are met. These include: (1) the occurrence at some point of interspecific matings, (2) that hybrids are produced which have lower but not zero fitness, and are selected against in the wild, (3) that variation is heritable and responsive to selection and (4) that reproductive character displacement has not evolved in response to other processes causing divergence in sympatry (Howard 1993; Rundle and Schluter 1998). mtDNA evidence of parental genotype and paternity testing indicates that interspecific matings and hybridisation occur in fur seals, although as already mentioned, whether hybrids have lower fitness remains to be established. These data satisfy the first and partially satisfy the second precondition for reinforcement. Demographic studies currently underway should provide data on the survival and breeding success of hybrids.

This study was unable to determine if reproductive character displacement was responsible for species recognition and mate choice among female fur seals, because we were not able to assess the phenotypic traits which were used by females to discriminate species. However, it is worth speculating on features which may be important, and which we feel merit further research. There is great uniformity in the appearance of the pelage in all species of fur seal, with the marked exception of the subantarctic fur seal. Males of this species are characterised by a yellowish chest, neck and face, with a top-knot or crest of dark fur on the forehead which becomes erect when the animal is excited (Condy 1978; Shaughnessy et al. 1988; Wilkinson and Bester 1990). These features alone have been considered enough to separate this species from all others in the genus (Bonner 1968; Repenning et al. 1971). Male vocalisations are the other obvious trait which could be examined. The key criteria for traits such as these to be considered as evidence of reproductive character displacement, as opposed to sympatric or allopatric divergence (Andersson 1994), are that the traits show greater divergence between species in areas of sympatry than in allopatry (Brown and Wilson 1956; Rundle and Schluter 1998).

One of the main problems in investigating possible character displacement in fur seals is that most populations were subjected to significant uncontrolled sealing in the late 18th and early 19th centuries, to the extent that many populations became locally extinct. The species distributions, and the sites where species were allopatric or sympatric prior to sealing is unknown and cannot be reconstructed with any accuracy. This is especially so for the Antarctic and subantarctic fur seals: for two of the three islands where these species currently breed sympatrically, the endemic populations were hunted to extinction. At the third site (Prince Edward Island) where a subantarctic fur seal population probably survived at the end of the sealing era, a small Antarctic fur seal population has established in recent years. It is possible that the highly distinct pelage pattern of the subantarctic fur seals evolved as a consequence of

character displacement among sites of secondary contact with Antarctic fur seal or other species during the radiation of the genus. The lack of distinctive pelage among others species of the genus may be evidence for allopatric speciation for most species in the genus, and speciation by reinforcement in the subantarctic fur seal. Whether divergent vocalisations between Antarctic and subantarctic fur seals have evolved through character displacement could be examined by comparing variation in the same vocalisations among species to see if the greatest divergence exists between Antarctic and subantarctic fur seals, compared with other species. This experiment would need to control for genetic distance between species.

One of the intentions of this study was to examine whether any conflicts existed among female fur seals at Macquarie Island between mate choice based on territory-holding ability (sexual selection) and mate choice for correct species. Our results indicate that such conflicts are unlikely: females have some capacity to discriminate between males both within and between species on phenotypic traits additional to those under sexual selection. Male territoriality alone does not guarantee mating success. Future studies should therefore seek to determine both the extent of female promiscuity and choice, the traits which females use to discriminate among males, as well as the importance of alternate male mating strategies.

Acknowledgements We thank Sabine Loew, Carl McIntosh, Eleni Paxinos and Beth Perry for help with analysis of samples. Kiren Lawton, Doreen Marchesan, Catherine Bone and Rosemary Whelan gave much assistance with field work on Macquarie Island. We thank Bill Amos, Mary Jane West-Eberhard, Steve Insley, Ted Miller, Tom Trogonza and Sally Troy for commenting on the manuscript. Biopsy samples were obtained from fur seals in accordance with the Australian Antarctic Animal Ethics Committee. We acknowledge the Smithsonian Institution, Friends of the National Zoo, the Australian Antarctic Science Advisory Committee and the Ian Potter Foundation for funding.

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Communicated by P. Thompson