

Lower reproductive success in hybrid fur seal males indicates fitness costs to hybridization

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Abstract

Hybridization among organisms can potentially contribute to the processes of evolution, but this depends on the fitness of hybrids relative to parental species. A small, recently formed population of fur seals on subantarctic Macquarie Island contains a high proportion of hybrids (17–30%) derived from combinations of three parental species: Antarctic, subantarctic and New Zealand fur seals. Mitochondrial control-region data (restriction fragment length polymorphisms) and nine microsatellites were used to determine the species composition of breeding adults, and hybrid male fitness was measured by comparing reproductive success (number of genetically inferred paternities) of hybrid and pure-species territory males over 6 years. No correlations were found between male reproductive success and three genetic measures of outbreeding, but this may be due to a relatively small number of dominant males analysed. Territory males fathered 63% of pups, but hybrid males had lower reproductive success than pure-species males despite having the same ability to hold territories. A greater proportion of females in hybrid male territories conceived extra-territorially than those in territories of pure-species males, and most (70 of 82) mated with conspecifics. This suggests the presence of reproductive isolating mechanisms that promote positive assortative mating and reduce the production of hybrid offspring. Although we found no evidence for male sterility in the population, mechanisms that reduce lifetime reproductive success may act to decrease the frequency of hybrids. Our study has identified a disadvantage of hybridization — reduced reproductive success of hybrid sons — that may be contributing to the persistence of pure lineages at Macquarie Island and the temporal decline in hybridization observed there.

Keywords: *Arctocephalus*, hybrid fitness, mean d^2 , microsatellite, paternity, reproductive isolation

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Introduction

Successful natural hybridization among higher animals was historically thought to be a rare phenomenon (Mayr 1963), but evidence for its occurrence is mounting. Hybridization has the potential to influence evolutionary processes such as speciation, but its role in evolution depends on the fitness of hybrids relative to parental species (Burke & Arnold 2001). Empirical studies of secondary

contact and hybridization between divergent lineages have documented broad variation in the fitness of hybrids. Many hybrids are less fit than parental species in parental habitats (e.g. ground crickets and topminnow species, Howard *et al.* 1998; Hurt & Hedrick 2003), but some have elevated fitness under particular environmental conditions (e.g. some trout species and finches, Grant & Grant 1992; Young *et al.* 2001). Fitness costs of hybridization can include reduced survival or fertility, and Haldane's rule states that when in the F_1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterogametic sex (Haldane 1922). In mammals, males are

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the heterogametic (XY) sex; so if Haldane's rule operates within a population, we may observe sterility in hybrid males.

The use of molecular techniques to study patterns of male reproductive success and mammalian mating systems has revealed that behavioural observations vary in their accuracy of reflecting paternity and the degree of polygyny (Pemberton *et al.* 1992; Amos *et al.* 1995; Goldsworthy *et al.* 1999; Worthington Wilmer *et al.* 1999; Gemmell *et al.* 2001; Lidgard *et al.* 2004; Twiss *et al.* 2006). Genetic analysis of paternity is therefore necessary to gain accurate insight into patterns of reproductive success, particularly in promiscuous mammals. Southern fur seal species (*Arctocephalus* spp.) breed terrestrially on oceanic islands or coastal beaches and many exhibit resource-defence polygyny (i.e. females aggregate in dense colonies and males defend areas of the beach and mate with many females within a season; Boness 1991). Females give birth shortly after arriving on the beach to a pup that was conceived 1 year earlier, and they remain with their pup until oestrus occurs approximately 7–9 days post-parturition. After parturition, females typically mate with a male before leaving the territory and their pup to forage at sea for several days. Territory males defend discrete areas of the beach that contain females, and by controlling access to females, males generally achieve more paternities than challenger or 'marginal' conspecifics that are relegated to territory boundaries where no females are in attendance (e.g. Hoffman *et al.* 2003). Mating rights within a territory are exclusive to the territory male and additional males within a territory are rarely tolerated; thus, the only way females can conceive with males other than the territory male is by moving out of their territory (Boness 1991). Because territory males defend a resource rather than females directly, during the mating season they maintain their positions and generally do not follow females, although attempts are made to retain females within their territory until mating has occurred.

Females provide all parental care for their offspring; therefore, it is expected that they should discriminate in their choice of a mate (Williams 1966; Trivers 1972), resulting in strong selection for traits that promote male competitive ability. Using reproductive success as an indicator of fitness in males is thus instructive, not only in terms of the potential for this sex to have reduced fertility as predicted by Haldane's rule, but also because fitness is governed by reproductive output. Further, male fur seals have a reproductive output that is highly dependent on their ability to defend and maintain female-occupied territories during the breeding season (Gentry 1980; Boyd 1989; Boness *et al.* 1993).

Previous studies examining the association between genetic relatedness and fitness traits including adult reproductive success have favoured three genetic measures of

individual inbreeding and outbreeding: (i) heterozygosity, (ii) internal relatedness (IR, a measure of relatedness of parental genotypes; Amos *et al.* 2001), and (iii) mean d^2 (the squared difference in repeat units between alleles at a microsatellite locus averaged across all loci at which an individual is typed; Coulson *et al.* 1998). Mean d^2 assumes the stepwise model of microsatellite mutation and that the distribution of alleles corresponds to coalescence times, with more highly divergent alleles presumed to have a common ancestor in the more distant evolutionary past (Pritchard & Feldman 1996; Coulson *et al.* 1999). While initially thought to be a good indicator of long-term inbreeding and outbreeding (Coulson *et al.* 1998, 1999; Marshall & Spalton 2000), mean d^2 is now thought to predict fitness best in specific situations such as under recent admixture of populations with substantial mutation rates, long divergence times and large subpopulation sizes (Tsistronis *et al.* 2001; Goudet & Keller 2002; Slate & Pemberton 2002). As yet, mean d^2 has been applied to few natural systems with these characteristics.

Macquarie Island in the Southern Ocean (54°30'S, 158°56'E) experienced local extinction of the original fur seal population (exact species composition unknown) due to over-harvesting in the 19th century (Csordas 1963; Ling 1999). Recent recolonization since the 1940s has been by three congeneric species: the Antarctic fur seal (*Arctocephalus gazella*), the subantarctic fur seal (*A. tropicalis*), and the New Zealand fur seal (*A. forsteri*). The level of hybridization in the population is high (17–30%) (Goldsworthy *et al.* 1999; Wynen 2001; Lancaster *et al.* 2006) relative to other sympatric colonies of southern fur seals (Hofmeyr *et al.* 2005; Kingston 2006). Although female New Zealand fur seals are absent from Macquarie Island and males of that species rarely hold territories (Shaughnessy *et al.* 1988), appreciable numbers of hybrid pups with genetic characteristics of New Zealand fur seals have recently been detected in the population, as have Antarctic–subantarctic (A–S) and Antarctic–subantarctic–New Zealand (A–S–NZ) hybrids (Lancaster *et al.* 2006). Despite hybridization among all three component species, the breeding population is far from homogenized. This may be due in part to assortative mating: males of all three species have different vocalizations and pelage characteristics that may enable conspecific recognition (Condy 1978; Page *et al.* 2001). The three species also tend to breed on different substrata: Antarctic fur seals generally breed on open, pebbly beaches; subantarctic fur seals more commonly breed in sheltered coves with large boulders; and New Zealand fur seals breed more often on large, angular rocks and rounded boulders (Condy 1978; Kerley 1983; Bradshaw *et al.* 1999).

The fitness distribution of hybrids in this population is currently unknown. Some degree of hybrid fertility is indicated by the presence of post-F₁ hybrids in the breeding population; however, the proportion of hybrids born has

decreased over the last decade (Lancaster *et al.* 2006). Whether this is due to fitness costs associated with hybridization or other biological factors is currently unknown. Therefore, we aimed in this study to assess and compare the reproductive success of hybrid and pure-species territory males to identify potential fitness costs of hybridization. In doing so the relationship between outbreeding (mean d^2 , heterozygosity, IR) and reproductive success was examined to assess empirically the performance of these measures, particularly mean d^2 , in the system.

Materials and methods

Study site and population

Eight complete annual cohorts of pups were sampled from Macquarie Island between 1992 and 2003 as described by Lancaster *et al.* (2006). Long-term monitoring of the fur seal population on Macquarie Island has included tagging of seals since the mid-1980s. These tags were used to identify adult males and females in the field, most of whom had skin biopsies taken when captured for tagging. Adult males present during the study period that did not have tags or identifiable markings were remotely sampled where possible with a barbed biopsy head attached to the end of a pole (Goldsworthy *et al.* 1999; Lancaster *et al.* 2006).

Species identification and measures of outbreeding

Genotypes and species assignments of all pups from Lancaster *et al.* (2006) were used in the current study (1007 pups from eight seasons: 1992, 1994–1996, 1998–1999, 2001, 2003). Adults (228 females, 54 males) were screened at nine microsatellite loci (all those reported in Lancaster *et al.* 2006 except Hg4.2) and a 417-bp fragment of the mitochondrial tRNA^{thr}-control region, and subsequently assigned to a pure species or hybrid class: pure Antarctic (A), subantarctic (S) or New Zealand (NZ) fur seal, or A–S, A–NZ, S–NZ or A–S–NZ hybrid (Lancaster *et al.* 2006).

The membership coefficient Q calculated in the Bayesian clustering software STRUCTURE version 2.0 (Pritchard *et al.* 2000) was used when assigning an individual as a pure species ($Q \geq 0.9$) or a hybrid ($Q < 0.9$) (Lancaster *et al.* 2006). The number of populations identified by STRUCTURE determined how many Q coefficients per individual there were; for Macquarie Island, three populations were identified (Antarctic, subantarctic and New Zealand fur seals) and validated with respect to definitive non-hybrid individuals from other islands (Lancaster *et al.* 2006). As such, individuals were ascribed a Q for belonging to each of the three species so that the three Q values for an individual summed to 1. Three measures of outbreeding were calculated to examine the association with territory male reproductive success: mean d^2 (standardized following Bean *et al.* 2004), IR

(Amos *et al.* 2001) and individual heterozygosity, H (Coltman *et al.* 1998). These measures were calculated for eight cohorts of pups and all adults (1288 individuals). Q_{\max} , mean d^2 , IR and H were tested for deviations from normality with quantile-quantile (Q-Q) plots and Kolmogorov–Smirnov one-sample tests, examined for homoscedasticity with Levene's test for homogeneity of variances and transformed where necessary.

Territory male reproductive success

To determine whether the territory male was the true father of a pup and hence to estimate territory male reproductive success, we required accurate field observations identifying all territory and challenger males and the territory location of females the year prior to pupping. This information was available for six years between 1991 and 1998. Pups are born one year after conception, so we analysed territory male reproductive success over the six years between 1992 and 1999, excluding 1993 and 1997. Any territory males in 1991 observed to hold territories in prior years were excluded from analyses as their prior reproductive success was not known. We henceforth refer to 'year' as the pupping (birth) year rather than the year of conception.

Parentage was inferred using the software program CERVUS version 2.0 (Marshall *et al.* 1998), which uses a likelihood-based approach to assign parentage to individuals based on codominant markers and a number of user-defined variables. For all analyses, allele frequencies were generated from all genotyped individuals in the population (pups and adults), the number of cycles was set to 10 000, the proportion of loci typed was 1.0 and the rate of typing error was 0.01. This latter parameter was derived from an empirical estimate from genotyping errors in mother–pup pairs matched in the field by calculating the genotype error rate for each locus and averaging over all loci (final rate of typing error of 0.009 per genotype; for detailed equations see Marshall *et al.* 1998).

Maternity

Of the 696 pups born between 1992 and 1999, 530 had putative mothers assigned to them in the field on the basis of nursing behaviour or physical proximity. CERVUS was used to confirm or reject parentage for these pairs (simulation parameters included 200 candidate mothers, 0.8 sampled). The 166 pups not assigned mothers in the field were tested against all 228 genotyped adult females as well as females known to have been born at least 4 years earlier (because females reach reproductive maturity at 3–4 years of age; Bonner 1994). Ideally, all females in a male's territory would be used in paternity analysis, but we were unable to obtain genetic samples from all females, so pups with no putative mother sampled were not

included. Paternity analysis was further restricted to pups with mothers that had known previous-year territory locations because the timing of oestrus for females that do not pup in a given year is unknown and many of these females are mobile during the breeding season. The spatial organization of territories held by hybrid and pure-species males was such that there should be no bias with respect to type of territory male in the distribution of sampled females and pups in the study population.

Paternity

Ninety-one skin samples from adult males present in the breeding colony over the 6 years were screened at all loci and 37 were found to be duplicate genotypes (detected using the matching program within GENALEX 6.0, Peakall & Smouse 2006). To ensure that these were duplicate biopsies (probably from males that were not tagged or otherwise identifiable among years) rather than multiple adult males with identical genotypes, the probability of identity was calculated using genotypes from all pups and adults. This approach estimates the average probability that two unrelated individuals drawn from the same population have the same genotype. The probability of this occurring in our samples was low [4.24×10^{-13} for unrelated individuals and 6.5×10^{-5} for siblings (GENALEX 6.0, Peakall & Smouse 2006)], indicating that repeated genotypes were likely to represent resampled individuals. Thus, a total of 54 adult males were included in the paternity analysis performed in CERVUS. The proportion of candidate males sampled differed among years and ranged from 0.6 to 0.8 (Table 1). Absolute reproductive success (total number of paternities achieved) was calculated for each territory male. Only paternities assigned to males with statistical confidence (80% and 95%) and no allele mismatches were included with the exception of paternity assignment to two territory males, where 'most likely' (ML) paternities were also included if their likelihood of paternity (LOD) scores were above 3.0 (Slate *et al.* 2000) and their genotypes did not mismatch with offspring. ML paternities are not assigned statistical confidence because the difference in likelihood between the first and second most likely candidate males is not sufficiently large. However, paternity assignments to both territory males were considered genuine because the likelihood (LOD score) of being the true father for every pup was high (between 4.0 and 8.0) and because the male next most likely to be the true father of each pup was the same in every instance. Given the high probability of identity in the population and few pairs of individuals sharing alleles at all loci, these pairs of males were likely highly related and thus affected the confidence with which true fathers were assigned paternity.

To test the hypotheses explaining variation in male reproductive success as measured by the number of sired

Table 1 Information on mother–pup pairs (M–P pairs) genotyped, proportion of candidate males sampled and paternity assignments across all years (where ML represents most likely)

Year	Proportion candidate males sampled	M–P pairs (N)	Territory male paternities (N)	Territory male paternities (%)	Confidence of assignment		
					95%	80%	ML
1992	0.6	26	17	65.4	5	3	9
1994	0.6	41	33	80.5	8	11	14
1995	0.7	55	37	67.3	23	4	10
1996	0.6	73	46	63.0	19	10	17
1998	0.7	75	44	58.7	28	11	5
1999	0.8	76	42	55.3	33	7	2
Total		346	219		116	46	57

offspring, we constructed a series of plausible generalized linear models (GLM) and compared them using an information-theoretic approach. With only 28 observations (males), we were cautious in building our model set to incorporate only the most logical hypotheses (six models only). All models included the number of breeding seasons as a control variable for the response (response = $\log[n + 1]$, where n = number of sired pups per male) because increasing the former will generally increase the latter. Next, we added the maximum value of Q assigned to each territory male (Q_{\max} ; see Methods) as an index of hybridization. Three models also considered mean d^2 in addition to Q_{\max} to examine whether this measure of outbreeding provided any more explanatory power to variation in fitness beyond that provided by Q_{\max} . Finally, two models also considered the number of adjacent territories around the focal male as an index of potential extra-territory paternities through movement of females into neighbouring territories.

We employed a multiple working hypotheses approach where models were contrasted based on Akaike's information criterion corrected for small sample sizes (AIC_c), which is an index of Kullback–Leibler (K–L) information loss (Burnham & Anderson 2002). This index is considered more appropriate to assess K–L information loss than other measures such as the dimension-consistent Bayesian information criterion when sample sizes are small (as was the case for our sample) (Burnham & Anderson 2004; Link & Barker 2006). Given that Q_{\max} is a proportion ranging from 0 to 1, we used the complementary log-log (clog-log) transformation to normalize the data. All GLMs were fitted using a Gaussian error distribution with an identity link (given the pretransformation of the response variable). Examination of the quantile–quantile (Q–Q) plots from the most highly parameterized models in the set demonstrated near-perfect normality after the log- and clog-log transformations of the response and Q_{\max} variables, respectively. We also estimated for all models the per cent deviance

Table 2 Species/hybrid composition of breeding adults across all years

Species/ hybrid identity	Territory males (N)						Adult females (N) All years
	1992	1994	1995	1996	1998	1999	
Pure	3	4	5	7	9	10	190
Hybrid	1	6	5	6	6	6	38
Total	4	10	10	13	15	16	228

explained (%DE) as a measure of goodness-of-fit. We used an information-theoretic evidence ratio (*ER*, an index of the weight of one model over another, calculated as the AIC_c weight (w) of the slope model/ w of the null model) (Burnham & Anderson 2002) to examine the relative statistical support of linear relationships.

Results

Species composition of breeding adults

The species composition of breeding females (mothers) and territory males differed. Females were mostly Antarctic fur seals (69.7%) while subantarctic fur seals and hybrids were less common (13.6 and 16.7%, respectively) (A: $\chi^2 = 19.81$, d.f. = 1, $P < 0.0001$; S: $\chi^2 = 8.28$, d.f. = 1, $P = 0.004$; hybrid: $\chi^2 = 5.37$, d.f. = 1, $P = 0.02$) (see Table S1, Supplementary material). In contrast, overall, territory males were comprised of similar proportions of Antarctic fur seals (37.5%), subantarctic fur seals (31.2%) and hybrids (31.2%), but the proportion of hybrid territory males differed among years and declined over time with the exception of 1992 (Table 2, Fig. 1). Ten out of 33 hybrid adult females and three hybrid males had *Q* values outside the range expected for F_1 hybrids (based on *Q* values obtained from simulation data in Lancaster *et al.* 2006) and were concluded to be post- F_1 hybrids and thus indicative of a degree of hybrid fertility.

Maternity assignment

Ninety-four per cent (497 of 530) of putative mothers assigned to pups in the field were genetically compatible at all microsatellite loci and mtDNA, and most maternities were confirmed with high statistical confidence (464 with 95% confidence; 29 with 80% confidence; 4 ML). Nineteen mother–pup pairs mismatched at a single locus, but only one pair persistently mismatched at a single allele after these individuals were rescreened and corrected. Fourteen (2.6%) of the mother–pup pairs assumed from field observations were incompatible as true parent–offspring at between two and eight loci. Seven of these had negative

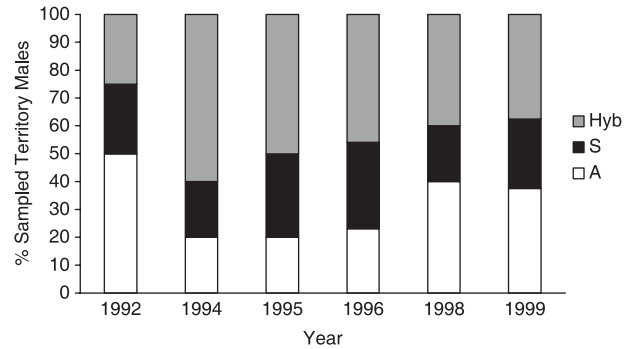


Fig. 1 Changing proportions of sampled pure-species and hybrid territory males in the population over time (A, Antarctic fur seal; S, subantarctic fur seal; HYB, hybrid).

LOD scores, implying that for these individuals, the field-assigned mother was less likely to be the true parent than an arbitrary unrelated female. The other seven had positive LOD scores but multiple mismatches, which remained after all pairs were rescreened. Based on these results, the best field-based error rate in matching mothers with pups was 1.3% (7 of 530 pairs), and at worst, it was 2.6%. Of the 165 pups not assigned to mothers in the field, CERVUS matched 15 with putative mothers with 80–95% confidence. Of all accepted mother–pup pairs (515 field-assigned/genetically confirmed, 15 genetically assigned only), 346 mothers had known previous year territories and were included in paternity analysis (Table 1). Remaining mothers either did not give birth the previous year or were not identifiable (untagged or unreadable tags).

Territory male reproductive success

The majority of males (20 of 28) held territories for a single year or two consecutive years; however, some returned for three or more years, and a small proportion for five and six years (Fig. 2a). All males that held territories over multiple years did so for consecutive years. The number of females in any one male's territory in a single year ranged from 1 to 25, with males known to have held territories for multiple years gaining access to more females overall. For the 346 mother–pup pairs where mothers were genotyped and had known territory locations the year prior to pupping, territory males were genetically identified fathers for 219 pups (63%, i.e. 116 with 95% confidence, 46 with 80% confidence and 57 ML) (Table 1). Variation in individual territory male success was large and ranged from zero to 38 paternities with a median of 2–7 (95% bootstrapped CI based on 10 000 iterations). Over half (16 of 28) of all territory males fathered between zero and five pups and nearly one-third of all paternities (66 of 218) were assigned to just two males (Fig. 2b).

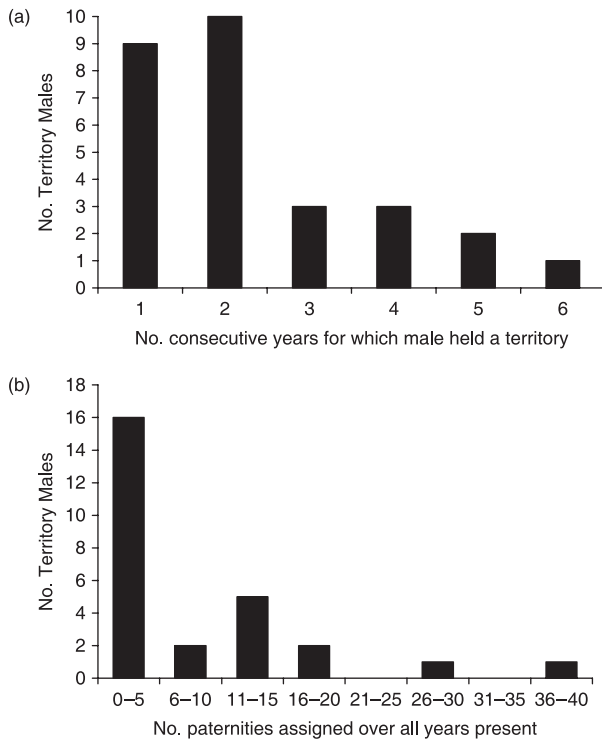


Fig. 2 Information on territory male (a) territory holding ability among years, and (b) individual reproductive success (total number of paternities achieved across all years present).

Extra-territory paternities (ETPs)

Territory males did not sire 128 of 346 pups (37%) born to females that resided in their territories in the year of conception, indicating that mothers of those pups mated with males other than the territory-holder after leaving their territory for foraging trips at sea. Of the 128 pups, 42 (12% of all pups and 33% of extra-territory paternities) were fathered by genotyped males who actively challenged territory males or were seen during the season. The remaining 86 pups mismatched with the most likely genotyped father at two or more loci and were thus considered to have been sired by unsampled males. This proportion of unresolved paternities (25%) is not surprising given that across all years only 60–80% of males in the population were sampled.

Hybrid male competitive ability

There was no evidence that hybrid males were any better or worse than pure-species males at gaining and holding territories within years. Across all years the proportion of hybrid males who held territories was similar to the proportion of hybrids in the overall adult male population, which included territory males, challengers and nonbreeding males ($\chi^2 = 0.02$, d.f. = 1 $P = 0.88$). There was also no

Table 3 Information-theoretic ranking of models testing the effects of degree of hybridization (Q_{\max}), mean d^2 (d^2) and the distance to adjacent territories to the focal male ($prox$) on reproductive success for 28 territory males. Also included in all models as a control variable is the number of breeding seasons (ssn) over which males sired pups (n). Log-likelihood (LL), number of parameters (k), rescaled Akaike's information criterion corrected for small sample sizes (ΔAIC_c), AIC_c weight ($wAIC_c$), and the per cent deviance explained (%DE) are shown for each model

Model	LL	k	ΔAIC_c	$wAIC_c$	%DE
$n \sim ssn + Q_{\max} + prox$	-20.260	5	0.000	0.784	76.7
$n \sim ssn + Q_{\max}$	-23.601	4	3.694	0.124	70.4
$n \sim ssn + d^2 + prox$	-22.849	5	5.178	0.059	72.0
$n \sim ssn + Q_{\max} + d^2$	-23.578	5	6.636	0.028	70.5
$n \sim ssn + d^2$	-26.961	4	10.415	0.004	62.4
$n \sim ssn$	-30.896	3	15.545	< 0.001	50.2

evidence for a difference between hybrids and pure-species males in the number of years over which they were known to hold territories ($t_{128} = -1.19$, $P = 0.26$), or the number of females in their territories. On average, pure-species males held territories for 2.1 years ($n = 18$), and each year had access to a mean of 9.7 females. Hybrids were territory males for an average of 2.8 years ($n = 10$) and had access to a mean of 11.8 females.

Lower reproductive success in hybrid males

Hybrid territory males were not sterile and all categories of hybrids investigated fathered pups, with the exception of the comparatively rare S–NZ hybrid type where only a single male held a territory for one year over the study period. The most parsimonious model (AIC_c weight = 78.4%) to explain deviance in male reproductive success (total number of paternities achieved) included the number of breeding seasons for which each male held a territory (control variable), the degree of hybridization (Q_{\max}), and the distance to the nearest neighbouring territory (Table 3). Mean d^2 strongly reflected outbreeding through hybridization, with pure Antarctic and subantarctic fur seals having lower mean d^2 than all types of hybrids, with only one exception (pure-species A compared to S–NZ hybrids, Fig. 3). However, there was only weak evidence that mean d^2 contributed to explaining variation in the number of pups sired beyond that already accounted for by Q_{\max} (model weight = 5.9%; Table 3). Approximately 20.2% of the deviance in male reproductive success was explained by Q_{\max} alone (after taking the number of breeding seasons into account), with an additional 6.3% explained by the distance to neighbours (Table 3). Q_{\max} was a much stronger predictor of male reproductive success than mean d^2 despite the relationship between the two terms ($ER = 7.7 \times 10^4$; $R^2 = 0.58$) (Fig. 4a). Further, when mean d^2 was replaced by

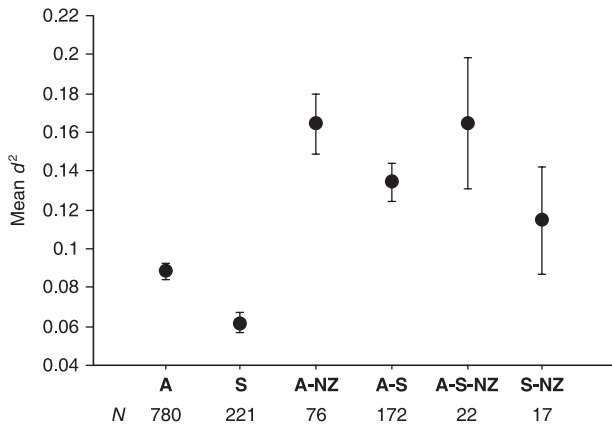


Fig. 3 Relationship between mean d^2 (mean \pm 2SE) and hybrid status in all pups and breeding adults, illustrating lower mean d^2 in pure species compared to hybrids. A, Antarctic fur seal; S, subantarctic fur seal; and NZ, New Zealand fur seal. Combinations (e.g. A-S) represent hybrids.

two other genetic measures; heterozygosity (H) and internal relatedness (IR), GLM results were identical (Fig. 4). The coefficient for Q_{\max} in the most highly ranked model was positive (1.25 ± 0.30), thus indicating a hybrid disadvantage; males with lower Q_{\max} sired fewer offspring than those with a higher probability of belonging to a single species. Because hybrid and pure-species territory males showed no difference in their ability to gain and hold territories in areas containing similar numbers of females, lower reproductive success observed in hybrid territory males is more likely due to reduced fertility in hybrid males or assortative mating/avoidance of hybrids by females.

Evidence of assortative mating

All extra-territory paternities (ETPs) were examined to establish whether females that conceived outside territories with males other than the territory male did so more frequently when the territory male was a hybrid, and whether the true father was conspecific to the female. In cases of mother-pup pairs where the true father was not sampled and the pup was not assigned a father, the species of the pup was used to establish the father's species. Thirty territories were compared: 11 held by pure-species males and 19 held by hybrids (Table 4). Other territories either had no extra-territory mating or there was considerable movement of females between close territories held by hybrid and pure males. ETPs from these territories were not included because the species of territory male could not be established unequivocally. A minimum of 31% of females (82 of 264) in territories held by hybrid males conceived extra-territorially compared to 14.5% (23 of 158) in territories held by pure-species males ($\chi^2 = 8.29$, d.f. = 1

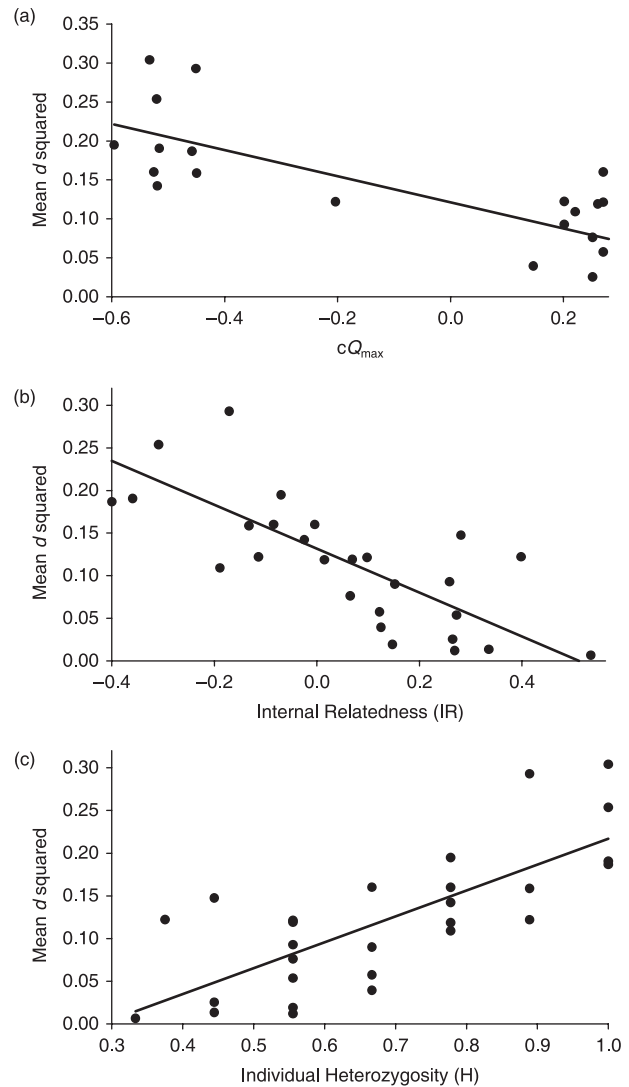


Fig. 4 Relationship of mean d^2 with (a) an index of hybridization, Q_{\max} (clog-log transformed) (evidence ratio [ER] = 7.7×10^4 ; $R^2 = 0.58$), and two measures of outbreeding; (b) internal relatedness (IR) ($ER = 1.5 \times 10^5$; $R^2 = 0.60$) and (c) heterozygosity (H) ($ER = 5.0 \times 10^4$; $R^2 = 0.56$).

$P = 0.004$) (Table 4). This conservative estimate is based on the total number of females in a male's territory (including unsampled females) and assumes that all pups of unsampled females were fathered by the territory male. Including only genotyped females from each territory, ETPs may be up to 24% (23 of 98) in territories held by pure-species males and as high as 60% (82 of 136) in territories held by hybrid males. Thus, even if we take into account a background level of ETPs (14–24% in conspecific territories), a higher proportion of females in territories held by hybrid males mated outside their territory ($\chi^2 = 26.6$, d.f. = 1 $P < 0.0001$). In most cases (70 of 82), those females that mated extra-territorially did so with conspecific

Table 4 Extra-territory paternities (ETPs) in territories held by pure-species and hybrid territory males (TM), showing evidence of assortative mating. A, Antarctic fur seal; S, subantarctic fur seal; and NZ, New Zealand fur seal. Combinations (e.g. A–S) represent hybrids

TM species (no. of territories)	Total females (<i>N</i>)	Sampled females (<i>N</i>)	ETPs (<i>N</i>)	ETPs conspecific to female <i>N</i> (%)
A (8)	104	72	18	17 (94.4)
S (3)	36	26	5	4 (80.0)
A–NZ (11)	156	84	45	38 (84.4)
A–S–NZ (6)	91	43	31	26 (83.9)
A–S (2)	17	9	6	4 (66.7)

males (Table 4). The exceptions were nine pure Antarctic females and three hybrid females that, despite mating extra-territorially, produced hybrid pups the following year. This indicates that they mated either with a hybrid or heterospecific challenger. These exceptions notwithstanding, hybrid territory males suffered higher frequencies of ETPs than pure-species territory males, with strong evidence that females that conceived extra-territorially bred with conspecific males.

Discussion

Establishing the fitness of hybrid offspring relative to their parental species is an essential precursor in understanding how natural hybridization operates in the process of speciation. If hybrids are less fit than parental lineages, in sympatry there may be reinforcement of reproductive isolation to complete barriers to interbreeding, but if no fitness costs are associated with hybridization, transfer of adaptations, merging of taxa and hybrid speciation may eventuate (Burke & Arnold 2001). We tested the fitness of hybrids in a polygynous natural system with a high level of hybridization among three species where male fitness is highly dependent on physical competitiveness and other phenotypic traits (McCann 1980; Clutton-Brock 1989; Boness 1991). We found that while hybrid males were similarly competitive as parental species in their ability to gain and hold territories containing females, they produced fewer offspring than pure-species males, illuminating an important disadvantage to hybridization for males in this system. This finding has major implications for understanding the mechanisms driving the temporal decline in hybridization frequency observed in the population (Lancaster *et al.* 2006) and the evolutionary importance of hybridization in general.

Several studies using pedigrees or modelling to analyse the usefulness of mean d^2 to detect fitness effects of inbreeding and outbreeding have found that mean d^2

outperforms other measures in only a limited number of circumstances (Hedrick *et al.* 2001; Tsistrone *et al.* 2001). Although the nature of the Macquarie Island fur seal population at least partially fulfils these circumstances (secondary contact between divergent populations with large population sizes), we did not find evidence of a significant association between male reproductive success and any of the three measures of outbreeding tested: mean d^2 , heterozygosity (H) or internal relatedness (IR), despite a strong relationship between mean d^2 and hybrid status. These findings are inconclusive at present due to the low number of dominant males in the population, as meta-analyses have shown that small sample sizes (< 50 individuals) may not have the power to detect associations between outbreeding and life-history traits such as reproductive success (Coltman & Slate 2003). At present, due to polygynous mating (many pups produced by a comparatively small number of dominant males) and the small size of the population, screening territory males and pups for more loci is possibly the only way to increase the sample size and improve the association of mean d^2 with male reproductive success in this system (Slate & Pemberton 2002 in Coltman & Slate 2003).

We found that territory males fathered 63% of all pups, a value similar to that determined in an earlier study of the same population (66%, Goldsworthy *et al.* 1999) and to Antarctic fur seals at South Georgia (59%; Hoffman *et al.* 2003). Proximity to neighbouring territories partially explained variation in reproductive success, with males that held territories farther from neighbours enjoying greater success. The presence of particular hybrid adult males in the breeding population (A–NZ, S–NZ and A–S–NZ) indicates a degree of male philopatry because only at Macquarie Island are New Zealand fur seals known to hybridize with Antarctic and subantarctic fur seals. These males represented 13% of all adult males sampled and a minimum of 19% of breeding males. In typical mammalian mating systems, females exhibit philopatry and males disperse; however, the closest breeding colonies of Antarctic and subantarctic fur seals to Macquarie Island are more than 5000 km away (Îles Kerguelen and Île Amsterdam), which may discourage dispersal by males. The presence of male philopatry in systems where females are also philopatric and both sexes exhibit strong site fidelity (Hoffman *et al.* 2006) is predicted to increase the likelihood of inbreeding. However, we found that the return of hybrid males to their natal site likely influences the degree of hybridization in the population rather than increasing the potential for inbreeding. Indeed, the decline in frequency of hybrid pups born in the population over time may be due partially to the proportional decrease in hybrid territory males observed during the later years of this study (1994–1999).

Hybrid and pure-species territory males were similarly able to gain territories, hold territories in areas containing

similar numbers of females, and return as territory holders for multiple years. However, comparison of extra-territory paternities revealed that more females in territories of hybrid males conceived with males other than the territory male, and in 85% of cases, the true father was conspecific to the female. This illuminates an important hybridization disadvantage to reproductive success over the lifetime of a hybrid male. Although some territory males fathered pups in years prior to when they held territories (data not shown), those paternities were not considered in this study as some males held tenure for the entire study period (6 years) so we could not establish their prior reproductive success. Lifetime reproductive success of some pure-species males investigated is therefore likely to have been slightly underestimated because those paternities were not included. However, because hybrid males rarely achieved extra-territory paternities, the number of pups they sired as territory holders is considered an accurate reflection of their lifetime reproductive success. Low reproductive success in hybrid males may be due to active avoidance of these males by females, sperm competition, or post-mating isolating mechanisms such as reduced compatibility of parental genes or genomes.

Females across many taxa are generally the more selective sex in interspecific encounters and often discriminate against heterospecific males (for examples, see Wirtz 1999). Goldsworthy *et al.* (1999) found that female fur seals could discriminate between males of different species based on phenotypic traits, and identified that the mechanisms by which females obtained extra-territory paternities was by leaving territories prior to oestrus, with females that left territories earlier more likely to obtain ETPs. However, their study did not identify hybrid individuals genetically, nor did it establish whether females who obtained ETPs did so with conspecifics. Our study significantly advances that of Goldsworthy *et al.* (1999) by using a comprehensive genetic panel to reveal that ETPs are also prevalent in territories held by hybrid males, and the high incidence of ETPs in territories held by hybrid males may result from a female's ability to distinguish pure-species males from hybrids, because ETPs were mostly with conspecific males. If extra-territory paternities resulted from active avoidance of hybrid territory males, a minimum of 29% of females chose to mate with conspecific challengers over hybrid territory males. This suggests that it may be more costly to mate with a hybrid territory male that exhibits good competitive ability than to mate with a conspecific challenger unable to hold a territory. If this is the case, having a hybrid pup must indeed be costly to female fitness. As suggested by Goldsworthy *et al.* (1999), a major potential barrier to hybridization among these three species is the difference in lactation length, which is 2.5 times shorter in Antarctic fur seals than the other two species (Boness 1991). Perhaps avoidance of hybrid males by female Antarctic fur seals is

due to the larger reproductive cost females may incur by having A-S or A-NZ hybrid offspring and being forced to wean them too early or expend energy lactating for longer. Further analysis incorporating female movement out of territories post-parturition and during oestrus is required to examine the hypotheses regarding active mate choice and avoidance of hybrid males in this population.

We have demonstrated that hybrid fur seal males are not sterile as might be observed under Haldane's rule. Haldane's rule also states that even if fertile offspring are produced initially, there will be a loss of fertility over multiple generations (Forsdyke 2000). We observed three hybrid territory males (10% of all males sampled) to be post-F₁ hybrids and all produced offspring. Four per cent of females sampled (10 of 228) were post-F₁ hybrids and all were fertile. With this small sample size, we cannot currently conclude that there is hybrid sterility or greatly reduced fertility over multiple generations that would result in strong selection against hybrids. Without definitive evidence for reduced fitness of hybrids and over such a short evolutionary timescale, there is limited support for assortative mating to have evolved through reinforcement in this population (reinforcement; Butlin 1995). However, we did find an important disadvantage associated with hybridization for males. Although it is still unclear which phenotypic marker traits females use to select mates, it is possible that hybridization creates new variation in such traits and hybrid males are recognized as less suitable mates. Pelage, odour and vocalizations have been suggested as potential characteristics under sexual selection, with vocalizations of hybrid males at Macquarie Island found to be intermediate to those of Antarctic and subantarctic fur seals (Goldsworthy *et al.* 1999; Page *et al.* 2001). If other traits are similarly affected by hybridization, this may explain why females prefer conspecific males if the alternative is to produce a hybrid son with phenotypic characteristics not favoured by females. Although this study did not focus on phenotypic traits that may be under sexual selection, reproductive character displacement in this population would provide more support for reduced fitness of hybrids and reinforcement of premating isolating mechanisms (Butlin 1995; Goldsworthy *et al.* 1999; Page *et al.* 2002). The persistence of pure fur seal lineages at Macquarie Island and the decline in hybridization observed both support assortative mating, and it is likely that this mechanism will contribute to driving the population towards purity over time.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Species composition of territory males, all adult males (territory males and challengers) and adult females across all years. A, Antarctic fur seal; S, subantarctic fur seal; and NZ, New Zealand fur seal. Combinations (e.g. A–S) represent hybrids.

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