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EXPLORING THE RELATIONSHIP BETWEEN PARENTAL RELATEDNESS AND MALE REPRODUCTIVE SUCCESS IN THE ANTARCTIC FUR SEAL *ARCTOCEPHALUS GAZELLA*

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Abstract.—Recent genetic studies of natural populations have shown that heterozygosity and other genetic estimates of parental relatedness correlate with a wide variety of fitness traits, from juvenile survival and parasite resistance to male reproductive success. Many of these traits involve health and survival, where the underlying mechanism may involve changes in the effectiveness of the immune system. However, for traits such as reproductive success, the likely mechanisms remain less obvious. In this paper, we examine the relationship between heterozygosity and a range of traits that contribute to male reproductive success, including time spent on territories and competitiveness. Our analysis is based on observational and genetic data from eight consecutive breeding seasons at a colony of the Antarctic fur seal, *Arctocephalus gazella*. Overall, male reproductive success was found to correlate strongly with internal relatedness (IR, a form of heterozygosity). When different components of success were analyzed, we found that IR correlates independently with reproductive longevity, time spent ashore, and competitive ability per unit mating opportunity on the study beach, with more heterozygous males being more successful. Behavioral observations were sufficiently detailed to allow examination of how daily mean IR values for males present on the beach varied within seasons and from year to year. Again, significant variation was found both among and within seasons, with more homozygous males appearing less able to hold territories in poor seasons when pup production is low and, within a season, at both the start of the season and to some extent around the peak of female estrus. Finally, we tested whether the benefits of high heterozygosity are due mainly to a genomewide effect (e.g. inbreeding depression) or to single locus heterosis by asking whether the relationship between IR and male success was robust to the removal of any single locus or to any pair of loci. Since the relationship remained significant in all cases, we favor a multilocus explanation for the effects we report.

Key words.—Breeding behavior, fitness, pinniped, male-male competition, microsatellite, heterozygosity, inbreeding, single-locus effect.

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It is well known that offspring of closely related parents often suffer from reduced fitness, or inbreeding depression. This arises because inbreeding increases homozygosity at genetic loci influencing fitness, thereby unmasking the expression of recessive deleterious alleles and/or reducing heterozygote advantage (Charlesworth and Charlesworth 1987). Inbreeding depression has long been of interest to evolutionary and conservation biologists because of its potentially profound influence on the evolution of mating systems (Charlesworth and Charlesworth 1987), the maintenance of genetic variation (Wright 1969), and the viability of small populations (Saccheri et al. 1998).

To date, most empirical data come from domestic or captive-bred wild species (for reviews, see Lacy et al. 1993; Falconer and Mackay 1996; Lynch and Walsh 1998) or from laboratory experiments involving the mating of very closely related individuals (e.g. Jiminez et al. 1994; Meagher et al. 2000). The study of natural populations is more challenging because, apart from a few exceptional studies (e.g. Keller et al. 1994; Reid et al. 2003), the deep pedigrees necessary for assessing inbreeding coefficients directly are not available. An alternative approach is to attempt to estimate parental relatedness using genetic markers. Using allozyme polymorphisms, a number of studies have revealed relationships between heterozygosity and fitness (e.g. Mitton and Grant 1984; Pemberton et al. 1988; Houle 1989). More recently, however, the discovery of highly polymorphic microsatellite markers and the development of new methods to estimate parental relatedness have both contributed to renewed interest in describing the relationship between inbreeding and fitness.

There are now three main methods for estimating parental relatedness using genetic markers. The simplest of these is to estimate the proportion of loci that are heterozygous. However, since genotyping is seldom complete, it is preferable to weight the contribution of each locus by the expected heterozygosity at that locus, to generate the measure standardized heterozygosity (Coltman et al. 1999). The second approach, mean d^2 (Coulson et al. 1998a), aims to go beyond classifying alleles simply as the same (homozygous) or different (heterozygous) by estimating how different heterozygous microsatellite alleles are in terms of their lengths. Averaged across many loci, mean d^2 estimates the evolutionary distance between the maternal and paternal genomes, exploiting the fact that microsatellites tend to mutate in a way such that the squared length difference between two alleles provides an estimate of the time to their most recent common ancestor (Goldstein et al. 1995). A third approach attempts to estimate the relatedness of an individual's parents

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using the extent of allele sharing relative to random expectations. This has been achieved in the measure known as internal relatedness (IR; Amos et al. 2001) by adapting the methods developed by Queller and Goodnight to measure relatedness between individuals (Queller and Goodnight 1989) to the special case of one allele per parent per locus.

Applied to the study of wild animal populations, these methods have shown that parental relatedness correlates with a variety of key fitness traits, including juvenile survival (Pemberton et al. 1988; Coltman et al. 1998; Marshall and Spalton 2000), adult survival (Keller et al. 1994), disease susceptibility (Acevedo-Whitehouse et al. 2003), and parasite burden (Coltman et al. 1999; Cassinello et al. 2001). Wherever these traits invoke parasites or disease, either directly or as factors that may make individuals succumb preferentially under environmental stress, the underlying mechanisms may involve any or many of the large number of genes that play a role in fighting infection. However, parental relatedness also impacts on reproductive success (e.g. Slate et al. 2000a; Amos et al. 2001; Höglund et al. 2002), and here the likely underlying mechanisms are perhaps less clear. Many of the ways in which males in particular may attempt to improve their fitness involve behaviors such as fighting, displaying, and territory holding that are based on traits such as aggression and attractiveness.

Since competition exacerbates inbreeding depression (Wolfe 1993; Haag et al. 2002), especially under stressful environmental conditions (Dahlgaard and Hoffmann 2000), the influence of parental relatedness on reproductive success could be especially profound in species with highly competitive mating systems where males fight to gain territories. This prediction is supported by experimental studies in mice (Potts et al. 1994; Meagher et al. 2000), which strongly implicate failure in male-male competition as a cause of the relatively low success of inbred males under seminatural conditions. However, little is currently known about the interplay between parental relatedness, territory-holding behavior, and reproductive success in natural populations.

The Antarctic fur seal *Arctocephalus gazella* provides an excellent opportunity to explore which of a number of possible ways parental relatedness may impact on male reproductive success in a natural mammalian population. The species breeds colonially, with males competing to hold territories among dense aggregations of the much smaller females (Bonner 1968). Breeding behavior is believed to be strongly polygynous, and although one recent genetic study calls this into question (Gemmell et al. 2001), a more extensive study in the same population confirms that male reproductive success is strongly skewed toward a few individuals (Hoffman et al. 2003). At Bird Island, South Georgia, one Antarctic fur seal colony has been studied since the 1950s (Bonner 1958). Access is provided by an aerial walkway above the colony (Doidge et al. 1984), allowing much greater ease of observation, marking, and sampling than at many other field sites. Genetic samples have now been collected for almost a decade, and the use of highly polymorphic microsatellite markers allows paternity assignment with great precision.

Here, we use observational and genetic data from eight consecutive breeding seasons to examine the relationship between parental relatedness, territorial behavior, and male reproductive success.

MATERIALS AND METHODS

The dataset and methods used in this study are largely identical to those described by Hoffman et al. (2003), the only major difference being an expansion of the number of breeding seasons covered by the study from seven to eight.

Study Site, Data Collection and Tissue Sampling

This study was conducted at Bird Island, South Georgia (54°00′S, 38°02′W) during the austral summers of 1994/1995–2001/2002 (hereafter, breeding seasons are referred to by the year in which they began). The study population was located at a small cobblestone breeding beach, separated from adjacent breeding sites by a cliff on the east side, open sea on the west, and rocky ridges to the north and south. The beach covers an area of 440m^2 at high tide (Lunn and Boyd 1993) and, on average, 649 pups were born there annually during the study period. An elevated scaffold walkway (Doidge et al. 1984) provides access to all parts of the beach, minimizing disturbance to animals.

To monitor the presence and locations of individuals, twice-daily surveys were made of all territorial males, females, and pups on the beach from 1 November until one week after the birth of the last pup or day 70, whichever came first. Since adult males are too large and aggressive to be captured and tagged, those occupying territories on the beach and on the surrounding rocks were individually marked using small patches of gloss paint (Arnould and Duck 1997). Paint marks were used for within-season identification and, in many cases, remained identifiable across years.

Samples were collected from pups by capturing them on the day of birth and collecting a small piece of skin from the interdigital margin of the foreflipper using piglet ear notching pliers (Majluf and Goebel 1992). Adult females were small enough to be captured and sampled in the same way later in the season (January–March). Territorial males were sampled using a biopsy dart system (Gemmell and Majluf 1997). All sampling equipment was sterilized using ethanol between uses. Skin samples were stored individually in the preservative buffer 20% dimethyl sulphoxide (DMSO) saturated with salt (Amos and Hoelzel 1991) and stored at −20°C. Table 1 shows the numbers of animals with unique genotypes present on the beach during the study period.

Total genomic DNA was extracted using an adapted Chelex 100 protocol (Walsh et al. 1991) and genotyped using a panel of nine dinucleotide-repeat microsatellite loci, as described elsewhere (Hoffman et al. 2003). Individuals that had been sampled more than once (within and/or between years) were identified using the program IDENTITY (Allen et al. 1995). Identity testing was particularly important to reveal males that had lost their paint mark identifications among and within seasons. After removal of identical multilocus genotypes, no locus showed significant deviation from Hardy-Weinberg equilibrium, nor was there evidence of linkage disequilibrium between any pairs of loci (Hoffman et al. 2003).

TABLE 1. Annual pup production, total numbers of adult males observed in the central study area grid, numbers of adult males with unique genotypes, and numbers of adult study females with genetically matching pups during 1994–2001. The total number of adult males includes unsampled individuals and excludes genotyped individuals that were found to be duplicates within seasons. The total number of study pups included in the paternity analysis is given in parentheses.

Year	1994	1995	1996	1997	1998	1999	2000	2001	Total
Pup production	584	797	732	501	693	444	696	747	5194
Total number of adult males observed	183	153	157	134	143	148	207	192	772
Number of adult males with unique genotypes	113	133	107	73	79	127	137	147	464
Number of adult females with genetically matching pups	109	102	126	82	72	56	113	105	277 (765)

Quantification of Male Reproductive Success

Male reproductive success was quantified by paternity testing each male against large numbers of pups sampled each season. To maximize the confidence level of paternity assignment, only pups whose mothers had also been sampled and genotyped were included. Sample sizes are given in Table 1. Paternity analysis was conducted using the programs NEWPAT XL (Worthington Wilmer et al. 1999) and CERVUS version 1.0 (Marshall et al. 1998; Slate et al. 2000b). These two programs yielded essentially identical results (Hoffman et al. 2003). Importantly, the exclusion probability was sufficiently high (0.999964) that virtually every paternity assignment was likely to be correct. This assertion was supported by the findings that among seasons only 9.2% of paternities were assigned to males in years they were not seen; within seasons only 6.8% of inferred conception dates fell outside the known tenure of the father, and most, if not all, of these cases may have been due to imperfect sighting records (Hoffman et al. 2003). A total of 461 paternities were assigned.

Estimation of Parental Relatedness

A number of measures have been developed for estimating parental relatedness using microsatellite data. These include heterozygosity, standardized heterozygosity (SH), mean d^2, and internal relatedness (IR). Several recent studies (Hedrick et al. 2001; Tsitrone et al. 2001; Goudet and Keller 2002; Slate and Pemberton 2002), including a meta-analysis of both published and unpublished studies (Coltman and Slate 2003), suggest that mean d^2 is unlikely to be as effective as heterozygosity, except in unusual circumstances such as the recent mixing of two dissimilar populations. Standardized Heterozygosity represents a logical improvement over simple heterozygosity where genotyping is incomplete. The difference between SH and IR is slight, with very high correlations across a range of species ($r^2 = 0.877$, $n = 464$ for this study, but see also Amos et al. 2001). However, IR is theoretically more informative, since it weights allele sharing by the frequencies of those alleles. This expectation is supported by studies in which direct comparisons have been made between IR and SH, which show that significant relationships between parental relatedness and fitness tend to be stronger when IR rather than SH is used as the measure (Amos et al. 2001; W. Amos, unpubl. data). Consequently, for this study we used IR, although analyses were repeated using SH and mean d^2 (which was in fact standardized by dividing each value by the maximum observed at that locus, thereby ensuring that each locus contributed equally; Hedrick et al. 2001) for comparison. All analyses were restricted to animals that were genotyped at eight or more microsatellite loci.

Internal relatedness requires the calculation of allele frequencies and it is not entirely clear whether these should be based on the entire sample set or, for example, a subset such as the adult males. Consequently, we examined the robustness of IR to the use of alternative estimates of allele frequencies. Internal relatedness values of the adult males calculated using allele frequencies from adult males alone were compared with values calculated using allele frequencies from our dataset of adult females. The resulting correlation was extremely high ($r^2 = 0.9988$, $n = 464$), indicating that small differences in the allele frequency distribution have a negligible impact on the calculation of IR.

Statistical Analyses

Statistical analyses were conducted using generalized linear models (GLMs) within the R package (Ihaka and Gentleman 1996). Analyses were restricted to individuals in the central study area grid (Table 1) where most if not all conceptions occur (Hoffman et al. 2003). For all models, distributions of standardized residuals around regressions were inspected to verify that they were normally distributed. First, we tested whether the genetic variables fitted alone explained significant variation in male reproductive success. Success was expressed as paternities per pup compared subsequent to first sighting of male, thereby excluding males seen only in the final year of the study, and was modeled using a binomial error structure. To compensate for overdispersion, significance testing was carried out using *F*-tests. We also examined the possibility of using total number of pups fathered as the response variable, but the resulting residuals were not distributed normally and too many males would have had to be excluded because of incomplete reproductive histories (i.e., all those males sampled in the first and last years of our study).

A full model of male reproductive success was then constructed in which explanatory variables fitted were the number of seasons in which males were sighted, the average number of days spent on the beach when estrus females were present (E_{ij}/Y where Y is the number of years seen and E_{ij} is the number of females who came into estrus on day j when the male being considered was present in year i), and male IR. Initially, the model was constructed with all terms fitted, including three-way interactions. Using standard deletion-testing procedures (Crawley 2002), each term was then

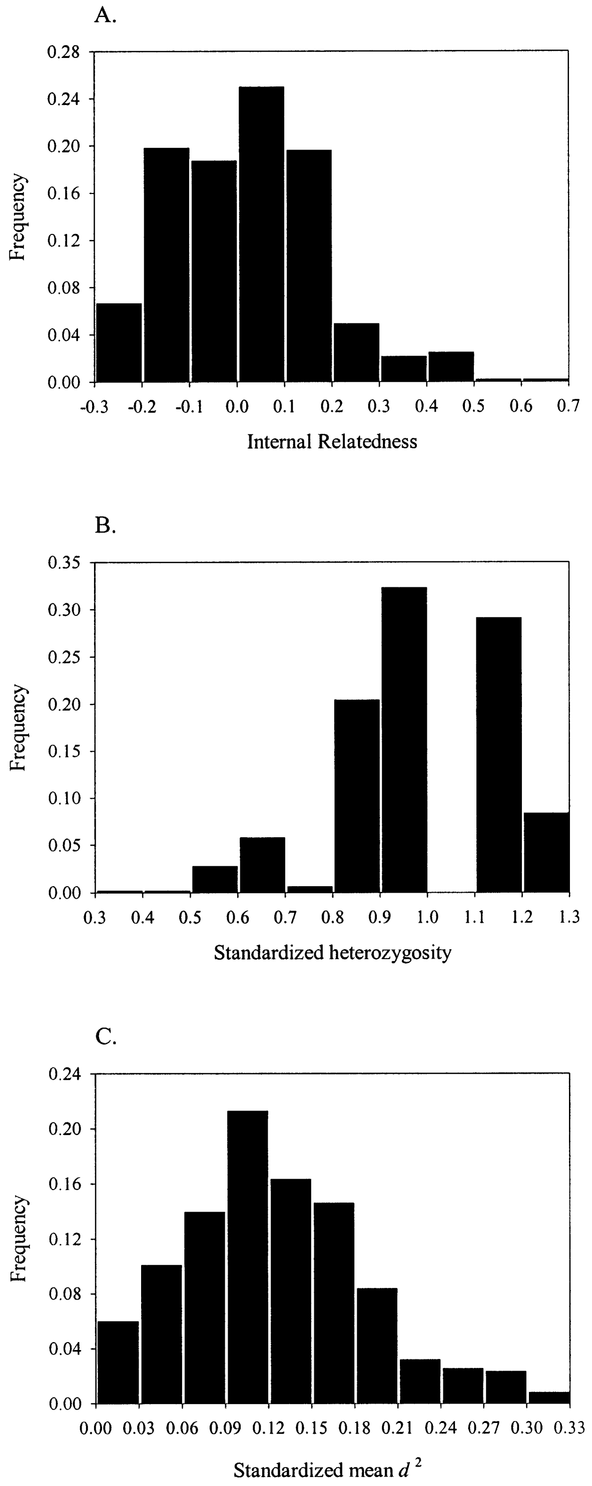

FIG. 1. Frequency distributions of (A) internal relatedness (IR); (B) standardized heterozygosity (SH); (C) standardized mean d^2 for 464 adult males. Since SH depends on the number of loci scored, there are some categories in the histogram for which there are very few counts.

TABLE 2. Correlation coefficients for the relationships between IR, SH, and standardized mean d^2 for 464 adult males. All correlations are significant at $P < 0.0001$.

Variable	IR	SH	Standardized mean d^2
IR	—	−0.937	−0.283
SH	—	—	0.335
Standardized mean d^2	—	—	—

dropped from the model unless doing so significantly reduced the amount of deviance explained (deviance is analogous to sums of squares in standard regression analysis). The change in deviance between full and reduced models was distributed as χ^2 with degrees of freedom equal to the difference in degrees of freedom between the models with and without the term in question. Because single-order terms cannot be removed from models if significant higher-order interactions are present, the significance of lower-order terms was assessed by comparison with reduced models not containing higher-order interactions.

RESULTS

During the course of this study, 464 males with unique genotypes were recorded on the central study area grid (Table 1), and these came ashore for between one and seven seasons and for between one and 62 days within a season. Paternity testing using the programs NEWPAT and CERVUS yielded highly congruent results and allowed us to assign a total of 461 paternities with little error.

Parental Relatedness

Until very recently, the majority of studies assumed that heterozygosity quantified at a small number of microsatellite loci reflects genomewide levels of inbreeding (the general effect hypothesis; Hansson and Westerberg 2002). In this paper we follow this convention, equating low parental relatedness (high heterozygosity) with being relatively outbred. In a later section we formally test whether this assumption is likely to hold true for our dataset. Parental relatedness was estimated for 464 adult males using IR, SH, and the standardized mean d^2 (Fig. 1). The frequency distributions of IR and standardized mean d^2 were approximately normal, but exhibited slight positive skew (skewness, IR = 0.68, mean d^2 = 0.55). SH was negatively and more strongly skewed (skewness = −0.88). Genetic variables were not transformed for the following reasons: (1) neither logarithmic nor arcsine-square-root transformation improved fit to a normal distribution; (2) GLMs are robust to somewhat skewed data (Genstat 5 Committee 1995); (3) The distribution of standardized residuals around the regressions between genetic variables and the trait of interest, male reproductive success, were distributed normally.

To determine the extent to which the three genetic variables were measuring similar properties, IR, SH, and standardized mean d^2 values were correlated against each other (Table 2). All of the correlations were statistically significant ($P <$ 0.0001), with IR and SH being the most strongly correlated.

Male Reproductive Success

To examine whether parental relatedness explains a significant proportion of variation in male reproductive success, we fitted a GLM. Male success was expressed as number of paternities assigned relative to the number of pups against whom that male had been tested in seasons subsequent to the first sighting of the male, and was modeled using a binomial error structure. Hence, males seen only in the final year of the study were excluded because their possible offspring had not yet been sampled. Both IR and SH correlated strongly with male reproductive success (IR $F_{1,390} = 19.44$, $P < 0.0001$; SH $F_{1,390} = 11.79$, $P < 0.001$), with relatively outbred males being more successful. In contrast, standardized mean d^2 did not explain significant deviance in success ($F_{1,390} = 2.17$, $P = 0.141$). Since the relationship was strongest for IR, we used this measure for subsequent analyses.

Male Reproductive Longevity

The simplest way outbred males might enhance their reproductive success is by coming ashore for more seasons, either by living longer or by being competitive over a longer period. Any estimation of male reproductive longevity is likely to be somewhat crude because, without a very long study period, there will always be uncertainty about whether any given sighting record is complete. In our sample set, males varied greatly in the number of seasons in which they were seen, but 76.7% were seen in only one or two seasons. Furthermore, 92.8% of males seen in two seasons and 79.5% of males seen in three seasons were sighted in consecutive years, indicating that males tended not to extend their active reproductive life by skipping seasons and then returning. Consequently, although we cannot help but underestimate the longevity of the top males, for most males our sighting records are likely to be a fair reflection of their staying power.

When all males were analyzed together, relatively outbred males appeared to come ashore for a greater number of seasons (GLM with number of years seen ashore modeled using a Poisson error structure, $\chi^2_{1,462} = 4.95$, $P = 0.027$). To obtain a more conservative estimate, the analysis was repeated after excluding all males seen in either the first or the last season, thereby minimizing the effect of histories that extend beyond the limits of this study. When this was done, the relationship, although retaining a similar slope, was no longer significant ($\chi^2_{1,202} = 0.56$, $P = 0.547$). However, the exclusion of males seen in 1994, the first year of genetic sampling, reduced the sample size by over 50% and removed many of the longest-lived males.

Interannual Variation in Male Territory Holding

A further complication was that not all seasons were equal because environmental variability causes large annual fluctuations in the availability of food. When food is scarce, females lose condition and many fewer return to the beach to give birth in the following season (Lunn and Boyd 1993). It seems likely that male behavior will also be affected, either directly through a similar loss of condition, or indirectly because the number of females is likely to affect the intensity of competition among males for mates. In practice, male numbers on the beach varied little across years. This may be because male behavior was less influenced by body condition or because, with a much smaller proportion of all males able to hold a territory ashore, any vacant territories were quickly occupied by the next male down the pecking order. However, even if the number of males varied little, this does not mean that there was no variation in quality.

To examine the extent to which male behavior exhibited interseasonal patterns, we plotted the mean IR of males seen ashore in each year against pup production, as a surrogate measure of season quality. Relatively inbred males were more likely to obtain territories in years when pup production was high (Fig. 2A, $P = 0.008$, $n = 8$, $r^2 = 0.717$). As an alternative measure of the ability of relatively inbred males to hold territories in years when female numbers were low, we also calculated the proportion of days in a season for which the average IR of territorial males was greater than zero. With

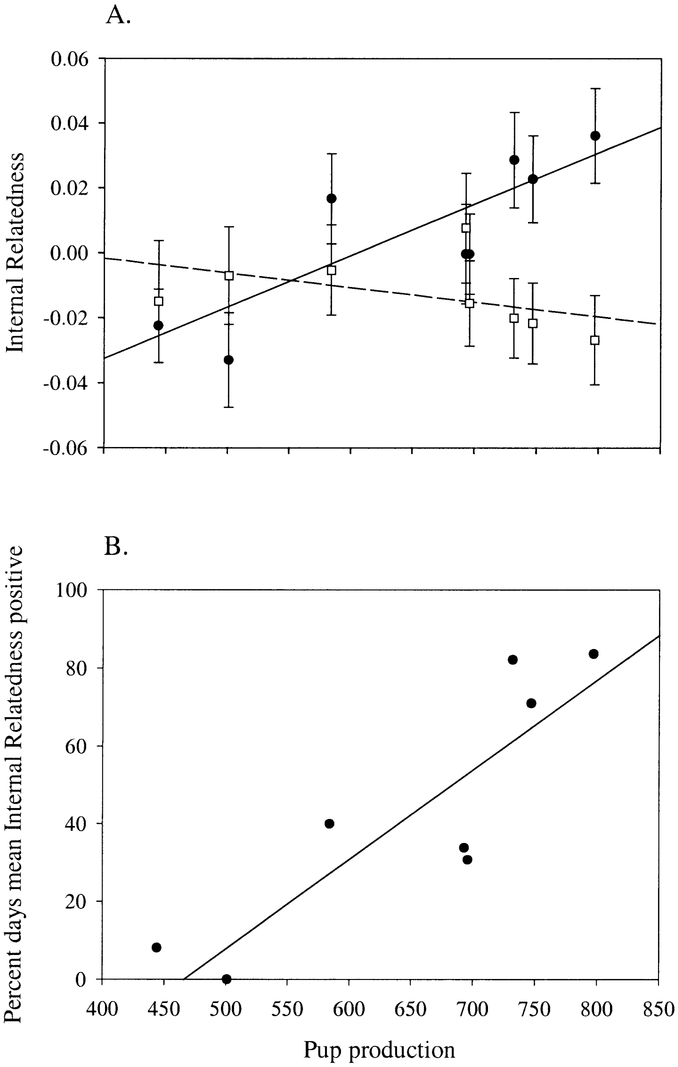

FIG. 2. Relationship between seasonal pup production and internal relatedness (IR) of animals present on the study beach. (A) Mean ± SE IR of territorial males (filled circles) and pupping study females (open squares). Fitted lines show linear regressions about the means. (B) Percentage of days during each season that mean male IR was positive.

TABLE 3. Generalized linear model of male reproductive longevity. Explanatory variables fitted were IR and the residuals around the relationship between IR and mean pup production of the years in which males were sighted (residual mean pup production). Only significant terms in the model are shown. The IR × residual mean pup production interaction was not significant. The percent deviance explained refers to the proportion of the total deviance explained by each term in the model. The χ^2 values for each term represent the change in deviance after removing that term and all interactions involving that term from the model.

$N = 464$, total deviance = 378.83, total explained deviance = 3.97%

Term	Estimate	% deviance explained	df	χ^2	P
IR	−0.479	1.30	1	4.84	0.029
Residual mean pup production	−0.001	2.67	1	10.20	0.001

this measure, the relationship was even stronger (Fig. 2B, $P < 0.005$, $n = 8$, $r^2 = 0.761$). Interestingly, during several seasons, the mean IR of territorial males was greater than zero. However, in fact the majority of paternities were assigned to males with IR values less than zero, suggesting that although holding a territory is a necessary condition for being successful, it may not be sufficient to gain paternities.

Thus far it is unclear which years were most stressful for a male. If male body condition were relatively unaffected by the food supply, low mean male IR values in seasons of low pup production might be interpreted as resulting from more intense male-male competition for a reduced resource, the receptive females. Conversely, if body condition was affected, years of high pup production may see males competing more intensely despite the increased resource. An indication of which of these explanations is closer to the truth can be gained by fitting the mean pup production of years in which males were seen ashore as an extra explanatory variable in the regression of longevity on IR. However, since IR and mean pup production were themselves correlated ($P < 0.005$, $n = 464$, $r^2 = 0.028$), we instead fitted the residuals of the relationship between these two variables. In the resulting model (Table 3), residual mean pup production was significantly negatively correlated with longevity, suggesting that years of high pup production were the most stressful to a male.

As a comparison with the male pattern, we also asked whether females with low IR values were more likely to come ashore in years when food was scarce. Regressing pup production on mean female IR, based on all sampled females that gave birth to pups on the study beach, we found that mean female IR did not vary significantly among years (Fig. 2A, $P = 0.354$, $n = 8$, $r^2 = 0.144$). This suggests that the ability of females to achieve the condition necessary to come ashore was not reduced by inbreeding and highlights a potentially important difference between the sexes.

Day-to-Day Variation in Male Territory Holding

The second way that outbred males might gain an advantage over relatively inbred conspecifics is by spending more time ashore within a season, especially during the period when most females come into estrus. To test this, we used detailed daily observations of the time over which males held territories to construct profiles of how mean male IR varied from day to day across each season (Fig. 3). Analysis of these data was complicated by strong nonindependence. Most males came ashore for several days at a time and some for more or less the entire season. Consequently, on consecutive days many of the same males were present. To provide an indication of the error limits that would result if male behavior was not influenced by parental relatedness, for each season we randomized male identity to generate daily expectations with appropriate 95% confidence intervals (±1.96 SD). We also included the distribution of numbers of females in estrus, with estrus dates being estimated as female parturition date plus seven days, the average postpartum delay before estrus (Duck 1990; Boyd 1991).

Regardless of the year, the first males to arrive tended to have low IR values. However, thereafter the daily mean male IR profiles varied greatly between seasons. When pup production was low (e.g. 1997 and 1999, Figs. 3G and 3H), daily mean IR of males remained consistently negative, lying near or outside the lower 95% confidence interval if IR had no influence on appearance. Conversely, when pup production was high (e.g. 1995 and 2001, Figs. 3A and 3B), mean IR was positive across most of the season, with peaks lying outside the upper 95% confidence interval if IR had no influence on appearance. Many of the profiles also appear to have a low point that embraces the front end of the peak of female estrus, flanked by regions in which IR is higher, particularly toward the end of the season (e.g. 1994, 1995, 1997, 2000, and 2001, Figs. 3F, 3A, 3G, 3D , and 3B respectively). The all-season average profile (Fig. 4) reflects these trends, starting low, rising sharply, and then declining gradually toward a second minimum at the front end of the peak of female estrus. Thereafter, the profile rises again toward its highest point near the end of the season.

If the double-peaked overall profile of male IR is genuine, we might expect the distribution of assigned paternities to follow a similar pattern, with low IR males dominating the peak and particularly the front end of the peak of female estrus. This prediction appears to be fulfilled since the mean IR of males assigned paternities (Fig. 5) correlates with the mean IR of males on the beach in the all-season average profile ($r_s = 0.426$, $n = 32$, $P = 0.015$). Furthermore, a significant correlation cannot arise unless there exists an underlying pattern, therefore we can conclude that both variables are distributed nonrandomly across the pupping season. Interestingly, apart from the very start of the season, the lowest IR values occur toward the front end of the female estrus distribution, and it is known that earlier pupping females tend to produce larger pups (Lunn et al. 1994; Boyd 1996). Our data thus provide tentative support for a model in which outbred males tend to dominate the part of the season

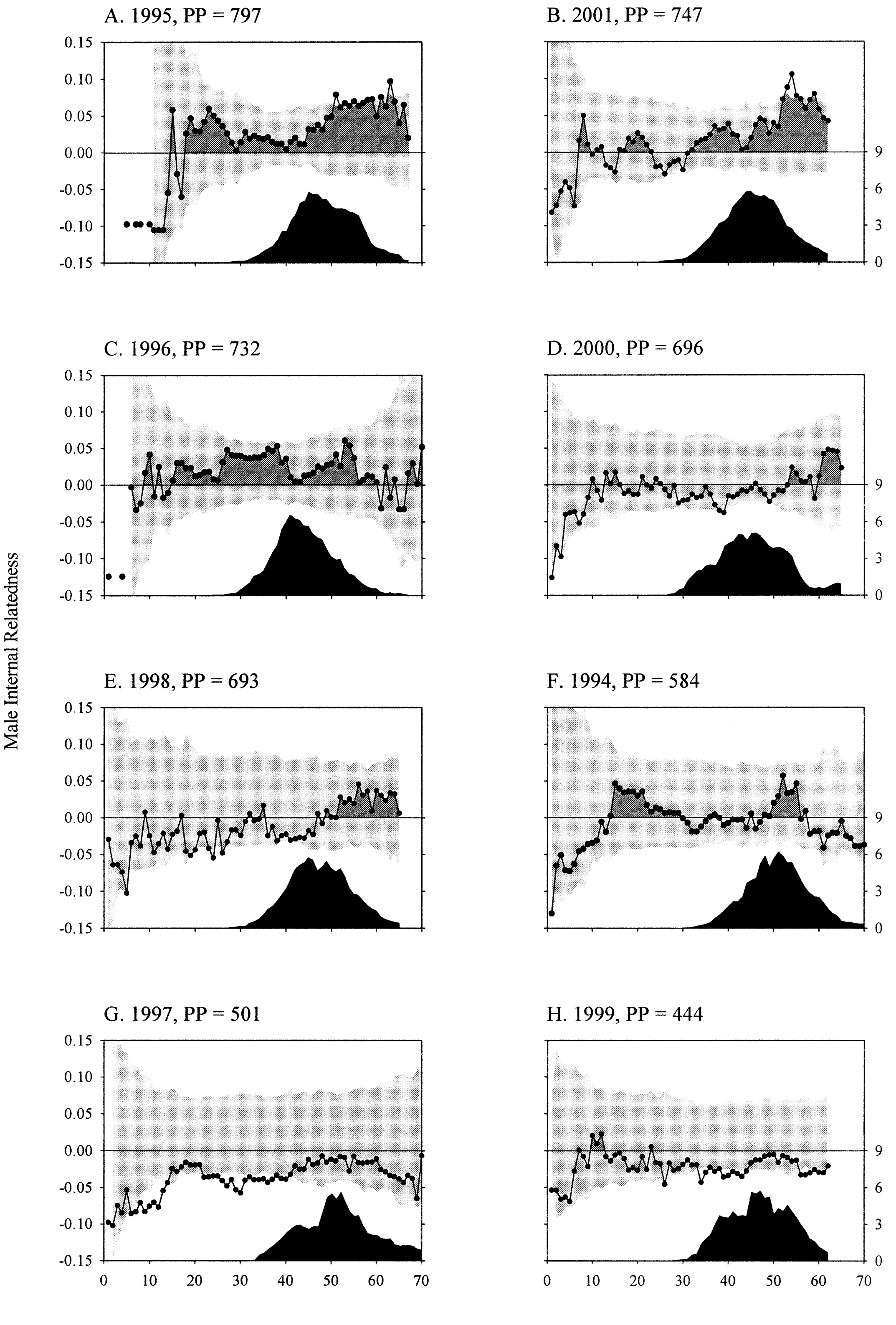

FIG. 3. Daily mean ± SE internal relatedness (IR) of males present in the colony relative to numbers of females in estrus. Individual years are presented in order of annual pup production (PP). 95% confidence intervals about daily average IR values (light gray shaded regions) were estimated using Monte Carlo simulation, to indicate the range of values expected should the timing and length of tenure be completely independent of a male's IR value. Dark gray areas indicate days on which IR was greater than zero and overlapped the simulation confidence interval; black area designates the profile of females in estrus (five-day moving average).

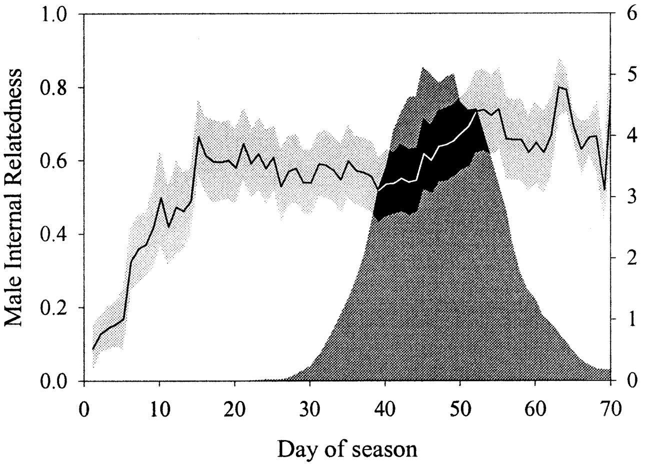

FIG. 4. Male internal relatedness (IR) profile averaged over all years, relative to the first day of each season. Data from different years were combined after expressing each daily mean IR value as a proportion of the minimum-to-maximum range in that year. The light gray region indicates the SE for the daily male average standardized IR. The profile of females in estrus is dark gray.

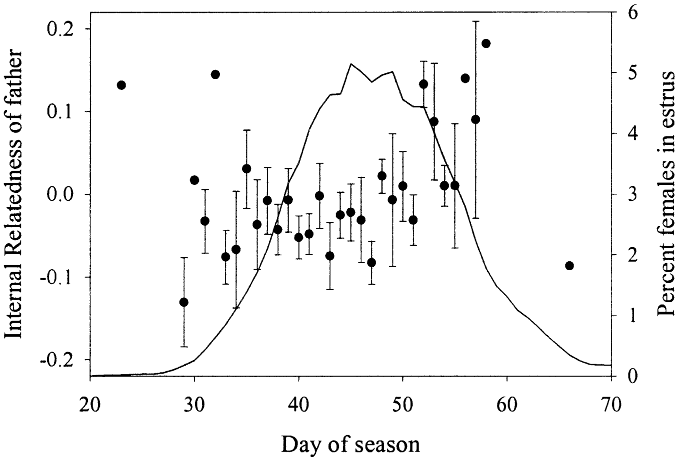

FIG. 5. Temporal profile of fathers' mean ± SE internal relatedness (IR), averaged over all years relative to the first day of each season. Data were restricted to 318 paternities, for which the assigned father was present on the estimated date of pup conception. Conception dates were estimated as female parturition date plus seven days. For reference, the profile of females in estrus is also shown.

when both the highest quality and greatest number of females come into estrus.

Relative Success Per Time Present in a Territory

The third way in which outbred males could improve their share of paternities would be by being more competitive or attractive such that they gain more success per unit opportunity on the beach. To examine this possibility, we fitted a GLM of male reproductive success, measured as pups fathered per pups compared and modeled with a binomial error structure, with primary terms number of years seen ashore, average number of estrus female days spent on the beach (E_{ij}/Y where Y is the number of years seen and E_{ij} is the number of females who came into estrus on day j when the male being considered was present in year i), and male IR. Both years and estrus female days were highly significant (Table 4), as seen previously. In addition, IR was independently significant at $P = 0.025$. Although the effect was not particularly strong, these data suggest that outbred males were disproportionately successful per unit time spent ashore.

Do All Loci Contribute Equally?

We have shown that Antarctic fur seal males with lower IR values (higher heterozygosity) tend to achieve increased reproductive success. Such a pattern could result in two ways (Hansson and Westerberg 2002). First, variation at microsatellite markers may reflect genomewide heterozygosity at unlinked genes exhibiting overdominance or segregating for deleterious recessive alleles that influence fitness (such an association is often termed a ''general effect''). Alternatively, one or more of the markers could be physically linked to loci that affect fitness (termed a ''local effect''). The former explanation predicts a genomewide effect, with the relationship between heterozygosity and fitness being more or less similar across microsatellite loci. In contrast, if the relationship were driven by a local effect, we would expect that it would be dependent on heterozygosity at a single locus.

To test whether the relationship between parental relatedness and male reproductive success could be due to a local effect, we ran the original GLM of male reproductive success again, fitting each locus individually and then dropping one

TABLE 4. Generalized linear model of male reproductive success. Explanatory variables fitted in the full model were IR, the number of seasons in which males were sighted (years), and the number of days present when estrus females were present, averaged over all years of each male's tenure (female days). Only significant terms in the model are shown. Three-way interactions and the IR × years interaction were not significant. The percent deviance explained refers to the proportion of the total deviance explained by each term in the model. The χ^2 values for each term represent the change in deviance after removing that term and all interactions involving that term from the model.

N = 392, total deviance = 898.58, total explained deviance = 32.95%

Term	Estimate	% deviance explained	df	χ^2	P
Years	0.447	15.94	2	89.59	<0.0001
Female days	0.145	12.92	3	133.42	<0.0001
IR	−1.918	2.16	2	7.36	0.025
Years × female days	−0.017	1.48	1	13.29	<0.001
IR × female days	0.123	0.45	1	3.99	0.046

TABLE 5. Generalized linear models of male reproductive success, fitting heterozygosity at each of nine microsatellite loci separately as the explanatory variables. The number of degrees of freedom (df) differs between models due to small numbers of missing single-locus genotypes. *P*-values are shown after Bonferroni correction for multiple tests (Hochberg 1988).

Locus	Estimate	Residual df	F	P
Aa4	0.112	387	1.12	0.954
Hg1.3	0.429	388	8.32	0.035
Hg6.3	0.262	380	4.29	0.294
Hg8.10	0.114	390	1.44	0.905
Lw10	−0.173	387	1.54	0.886
M11a	1.037	385	20.47	<0.0001
Pv9	0.145	388	1.43	0.907
PvcA	0.139	389	1.57	0.879
PvcE	0.068	389	0.27	0.999

TABLE 6. The influence of single loci on the relationship between IR and male reproductive success, evaluated by re-running the original GLM after dropping each locus in turn from the IR calculation. *P*-values are shown after Bonferroni correction for multiple tests (Hochberg 1988).

Locus	Estimate	Residual df	F	P
Aa4	−1.346	390	19.10	<0.001
Hg1.3	−1.044	390	12.97	0.003
Hg6.3	−1.176	390	15.29	<0.001
Hg8.10	−1.249	390	15.93	<0.001
Lw10	−1.344	390	22.95	<0.0001
M11a	−1.002	390	12.33	<0.004
Pv9	−1.276	390	18.49	<0.001
PvcA	−1.142	390	15.00	<0.001
PvcE	−1.467	390	22.78	<0.0001

locus at a time from the calculation of IR. To compensate for non-normality in single-locus IR values, we fitted heterozygosity (as the categorical variable with heterozygotes scored as one and homozygotes scored as zero) as the predictor variable in single-locus models.

Fitting each locus individually (Table 5), the slope of the relationship between heterozygosity and male reproductive success was positive for all but one of the loci employed, which is significantly more than would be expected by chance (sign test, $P < 0.05$). Such a pattern is thus consistent with a general effect of increasing success with heterozygosity. Interestingly, however, following Bonferroni correction for multiple tests (Hochberg 1988), we found that heterozygosity at two individual loci, M11a and Hg1.3, explained significant deviance in male reproductive success. When all of the loci were fitted as explanatory variables in a single model, the same two loci were again found to be significant, although the F-value for locus M11a was this time lower ($F_{1,385} = 16.17$, $P < 0.0001$). These results suggest that two particular loci appear to contribute disproportionately to the observed relationship between IR and success.

After removing any single locus from the calculation of IR, the relationship between IR and male reproductive success remained both negative and highly significant (Table 6). Even following a conservative Bonferroni correction for multiple tests, all correlations remained significant at $P < 0.005$. However, since two loci explained significant deviance in male reproductive success when fitted alone, we then ran the GLM again after removing both of these loci from the calculation of IR. Again, the relationship between IR and reproductive success remained negative and significant ($F_{1,390} = 7.10$, $P = 0.007$).

Following a suggestion from an anonymous referee, we also examined all possible pairwise correlations of heterozygosity among all loci individually, to see whether there was an excess of positive associations. Three of the 36 Spearman rank correlations were significant at $P < 0.05$, although none of these remained significant after Bonferroni correction. Of the 36 correlations, 17 were positive and 19 negative, a ratio that does not differ significantly from 50:50 (sign test, $P = 0.868$). However, it is unclear whether this test has any real power. In a larger study based on 71 loci in a small, isolated population of red deer with extreme polygyny, Slate and Pemberton (2002) only detected a marginally significant positive correlation across loci (1294 of 2485 comparisons positive, $P = 0.043$). Elsewhere, extensive stochastic simulations have been used to assess the extent to which heterozygosity is expected to be correlated among loci, and these show that although independent panels of 100 loci may correlate in mean heterozygosity, if panel size is reduced to 10 (let alone one), the tendency is lost in all but unrealistically extreme scenarios (Balloux et al. 2004).

DISCUSSION

We analyzed a large behavioral and genetic dataset of Antarctic fur seals in an attempt to discover whether parental relatedness influences male reproductive success and, if so, how. Males with high heterozygosity who are presumed to be relatively outbred did indeed father more pups than equivalent males with lower heterozygosity. Higher success appears to be achieved through a combination of factors, including greater reproductive longevity, earlier arrival within a season, and greater presence around the peak of female estrus. In addition, after controlling for differences in the time that males spent ashore, individuals with high heterozygosity also appeared more competitive, fathering greater numbers of pups per unit opportunity.

A number of recent studies indicate that genetic measures of parental relatedness such as heterozygosity, standardized mean d^2, and IR are correlated with a range of fitness measures (e.g. Coltman et al. 1998, 1999; Coulson et al. 1998a, 1999; Marshall and Spalton 2000; Slate et al. 2000a; Amos et al. 2001; Höglund et al. 2002; Schiegg et al. 2002; Slate and Pemberton 2002; Acevedo-Whitehouse et al. 2003; Reid et al. 2003). Our data lend further support to this trend, revealing a pattern in which males born to less-related parents tend to father more pups. Since mean d^2 increasingly seems to be effective only under certain highly restricted circumstances, we focused on SH and IR. Both these measures revealed similar trends of similar strength though here, as elsewhere, the trends were a little stronger with IR and hence this is the measure we preferred.

Previous studies have found correlations between genetic measures of parental relatedness and juvenile survival, tolerance of toxins, and resistance to parasitic infection and other diseases (Pemberton et al. 1988; Coltman et al. 1998, 1999; Cassinello et al. 2001; Acevedo-Whitehouse et al.

2003). Consequently, the most direct way in which heterozygosity might become linked to male reproductive success would be if relatively inbred males died younger and hence had less opportunity to mate. Our data support this possibility, showing that males with high IR values (low heterozygosity) come ashore on average for fewer seasons. Although significant, the relationship is not particularly strong, and it is not yet clear whether this is because the true relationship is weak, or because our measure of longevity has too many inaccuracies. In particular, since most males come ashore for only one or two seasons, greatest leverage is associated with the minority of males who achieve three or more seasons. Unfortunately, the longer the reproductive history, the greater the chance that part of it lies outside the range of our study, and hence the less accurately it is determined. The importance of these longer-lived males is emphasized by the loss of significance when males seen first in 1994 were excluded from the analysis.

A further complication in assessing male reproductive longevity was that there is great year-to-year variation in the number of receptive females present on the study beach, with high years seeing almost twice as many females as low years. Consequently, we tested the hypothesis that relatively inbred males would be less likely to obtain territories during years of low pup production. Two different measures of the ability of inbred males to come ashore plotted against pup production both revealed positive relationships, with males being more outbred in years when there were fewer females. Such a pattern could reflect two nonexclusive mechanisms. On the one hand, when females were scarce, male-male competition may have been more intense, resulting in lower quality males being excluded from the beach. On the other hand, the ability to come ashore may have reflected body condition generated in the months prior to breeding. If this were the case, good years could see more inbred males reaching the required body condition to come ashore, and hence the intensity of fighting on the beach could be increased. To test these opposing predictions, the relationship between IR and reproductive longevity was reanalyzed, fitting the mean pup production of years in which males were seen as an additional factor. Years of high pup production were found to negatively impact male reproductive longevity. Although further work is needed, this suggests that male competition may be divided into two components, one involving gaining access to the beach and a second relating to interactions once a territory has been obtained. Of these, the latter appears to have a longer lasting impact on male longevity.

The finding that a male's ability to come ashore was influenced by both parental relatedness and seasonal environmental conditions is consistent with a number of studies demonstrating that interactions between genetic and environmental heterogeneity may affect fitness (e.g. Keller et al. 1994; Coulson et al. 1998b). In contrast, females did not show a similar trend, suggesting that whatever factors determine whether a female gives birth in any year are not impacted by parental relatedness. Previous work has suggested that inbreeding depression may influence the two sexes differently (Coulson et al. 1999; but see Slate and Pemberton 2002). In the Antarctic fur seal, the difference between the sexes may well reflect the fact that males compete for space on the beach whereas females do not, especially since male-male competition has already been implicated as a factor that may amplify the detrimental effects of inbreeding depression under experimental conditions (Potts et al. 1994; Meagher et al. 2000).

The collection of daily observations of male presence on the study beach allowed a fine-scale temporal analysis of how male presence related to parental relatedness. The resulting plots of daily mean IR values were complicated, but revealed a number of features. One of the strongest trends was the one already suggested by the among-year analysis, in that the proportion of days over which the mean IR value was above zero ranged from zero in low pup production years up to almost 100% in high pup production years. A second clear trend observed was for the males with very low IR values to arrive early, and this was consistent across all seasons regardless of pup production. Although this suggests that relatively outbred males were the first to establish territories, an alternative explanation could be that these individuals are immigrants from outside the study area who carry unusual alleles. However, IR and SH were highly correlated (Table 2), and the initial dip remains regardless of which method we use (data not shown), suggesting that the high heterozygosity of these males is genuine and is not due simply to their coming from a different gene pool. Indeed, since many of these early males father pups on the beach, genetic differences between them and the other animals on the beach are likely to be small if they exist at all. Consequently, our results support a variety of nongenetic studies suggesting that males of the highest phenotypic quality are often the first to arrive at breeding grounds (e.g. Møller 1994, 2001; Marra et al. 1998). Such individuals need to be in good condition to maintain control of their territories until females arrive, but early arrival typically confers many advantages, including the acquisition of preferred territories and prior resident advantage in subsequent territorial contests (Maynard Smith and Parker 1976; Davies 1978; Riechert 1979; Holberton et al. 1990; Haley 1994; Cutts et al. 1999).

Other trends in IR across the season were more suggestive. In the profile of IR with time through the breeding season averaged across all seasons, mean IR increased sharply after the early arrivals and then appeared to gradually decline toward a second low around the early side of the peak of females' estrus. Thereafter, mean IR increased again toward a more defined peak at the end of the season. Such a profile would be consistent with two phases of competition, the first being at the start of the season and involving a small number of top males seeking to occupy the best sites, and the second could represent competition to hold territories at the prime time when most females come into estrus.

Interestingly, the peak of estrus dip in IR seen among males present on the beach appeared to be mirrored in the distribution of IR values among males that were assigned paternity. Specifically, males fathering pups when most females were becoming receptive tended to be more outbred than males that fathered pups either earlier or later. There was also an indication that both of these low points did not coincide exactly with the peak of estrus, but instead appeared to be shifted toward the early side of the peak. Independent data indicate that this is when heavier pups are conceived (Lunn et al. 1994; Boyd 1996), raising the possibility that males are competing both for the number of available females and for higher quality females capable of producing heavier pups.

The final aspect of male success that we examined was the ability to gain disproportionate numbers of paternities for any given opportunity. We found that, even after controlling for reproductive longevity and the number of estrus female days spent ashore, males with lower IR values tended to be more successful than those with higher values. At present, we do not know whether this increased success reflects differences in attractiveness to females, possibly mediated through olfactorily detected MHC diversity, or to an ability to occupy higher quality territories. To distinguish between these alternatives, we are currently increasing our sample size so as to construct accurate spatial maps relating territory size and position to the numbers of pups conceived. We also plan to genotype animals for MHC polymorphisms to examine the extent to which aspects of any mate choice present depends upon MHC alleles.

Although the full model of reproductive success explains almost one-third of the total trait deviance (Table 4), IR explains little more than 2% of the total deviance, a figure that is consistent with other similar studies. For example, Amos et al. (2001) examined four separate systems and found that about 2% of the total variation in reproductive success was explained in each case (exact figures not reported). Slate et al. (2000) report about 5% and Merilä et al. (2003) about 11%, though the latter is a special case in which hybridization is thought to be occurring. At face value, these figures appear small, but the true size of effect could well be substantially stronger. A panel of approximately 10 markers yields an extremely poor estimator of genomewide heterozygosity. Extensive simulation analyses indicate that if a panel of about 300 markers were available, the resulting decrease in variance of the estimate of individual heterozygosity would cause a corresponding increase in the proportion of variance in fitness explained on the order of 10-fold (Balloux et al. 2004; W. Amos, pers. obs.). The exact value depends critically on the exact distribution of *f*-values in the population, which in turn reflects the distribution of male reproductive success, the strength of any inbreeding avoidance behaviors, and the degree of genetic interchange between colonies, parameters that are currently undetermined. A similar but by and large much smaller increase in the proportion of variance explained might also result if we could improve our estimates of male success, for example by sampling a much higher proportion of pups on the beach.

Throughout this manuscript we have followed many similar studies by equating high heterozygosity with being relatively outbred. The unstated assumption is that heterozygosity quantified at a small number of microsatellite loci reflects genomewide levels of inbreeding (the general effect hypothesis; Hansson and Westerberg 2002). Limited support for this hypothesis comes from a comparison of genetic and pedigree estimates of inbreeding in a small captive wolf population (Ellegren 1999), which found a strong relationship between heterozygosity at 29 microsatellite loci and Wright's f ($r^2 = 0.67$, $P < 0.0001$). In addition, a study using more than 70 markers by Slate and Pemberton (2002) detected significant relationships between birth weight in red deer and heterozygosity using multiple sets of 10 loci. However, these two studies both involve unusual scenarios, in one case a very small captive colony in which inbreeding is forced and the other a highly polygynous species in which again, close relatives will often meet each other. In many natural populations, the overwhelming majority of individuals are likely to have inbreeding coefficients at or very close to zero, and hence heterozygosity, regardless of the number of markers used, will have little or nothing to do with inbreeding. In view of this, increasing attention is being focused on possible associations between neutral markers and loci experiencing balancing selection. For example, Hansson et al. (2001) found that microsatellite heterozygosity in great reed warblers predicted recruitment success among individuals of equal inbreeding coefficient, and Merila et al. (2003) identified a strong association between heterozygosity at a single microsatellite locus and male reproductive success in collared flycatchers. Simulation studies support this pattern, indicating that, unless there is strong polygyny and/or population substructure, heterozygosity will be relatively uncorrelated both among loci and with inbreeding coefficient (Balloux et al. 2004).

Our current study appears to lie between the extremes of Hedrick et al.'s (2001) captive wolf study and large homogeneous populations in which inbreeding is effectively absent, in that our fur seals appear to show strong philopatry to a rather small breeding beach and variance in male reproductive success is high. Whether these conditions are sufficient to create large variation in *f* and hence a strong link between heterozygosity and inbreeding coefficient is unclear. Consequently, we tried to determine whether inbreeding depression or heterosis is the more important factor in our study by testing each locus in turn for a link with male reproductive success. All but one of the loci show a positive association between heterozygosity and success, and two (M11a and Hg1.3) show significance at $P < 0.05$ following Bonferroni correction (Table 5). However, even after removing these two single-effect loci from the calculation of IR, the relationship between IR and success remained highly significant ($P = 0.007$). Our data thus do not support a model in which the entire pattern is driven by only one or two loci. Instead, the most parsimonious explanation for the correlations we observe seems to be a genomewide effect, that is, inbreeding depression. If single-locus effects do play an important role, then they would have to be operating at many independent loci, probably more than seem reasonable based on current knowledge. It is important to stress that the animals in our study probably show unusually large variation in *f*, and that the effects we describe may be substantially weaker in other species in which levels of philopatry and polygyny are lower.

In conclusion, we set out to explore whether genetic measures of parental relatedness correlate with measurable components of male reproductive success. Remarkably, each of the aspects we examined, namely number of seasons spent ashore, the distribution of tenure within each season, and some aspect of competitive ability over and above temporal domination, all correlate with IR. Consequently, our study extends the range of fitness-related traits in natural populations that reveal dependence on heterozygosity at presumed neutral microsatellite loci. Our analyses also suggest that temporal aspects appear to be an unexpectedly important determinant of male reproductive success in the Antarctic fur seal. In breeding systems where males hold territories, mating tactics are often viewed as a discrete choice between dominance and ''sneaking'' (Dunbar 1982; Gross 1996). Our findings suggest that the true picture may instead involve more of a continuum of states, with success depending upon access to the beach, which in turn varies greatly both among and within seasons. Further work will be required to determine unequivocally the relative importance of genomewide and local effects, but this represents an interesting avenue for future research.

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