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LETTERS

Female fur seals show active choice for males that are heterozygous and unrelated

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Much debate surrounds the exact rules that influence mating behaviour, and in particular the selective forces that explain the evolution of female preferences. A key example is the lek paradox, in which female choice is expected rapidly to become ineffective owing to loss of additive genetic variability for the preferred traits[1–3]. Here we exploit a remarkable system in which female fur seals exert choice by moving across a crowded breeding colony to visit largely static males. We show that females move further to maximize the balance between male high multilocus heterozygosity and low relatedness. Such a system shows that female choice can be important even in a strongly polygynous species, and at the same time may help to resolve the lek paradox because heterozygosity has low heritability and inbreeding avoidance means there is no single 'best' male for all females.

Females usually invest much more in offspring than males and are therefore expected to be choosy, for instance favouring partners with good genes[4]. However, despite many documented examples of female choice[5], the link between choosiness and offspring quality is not always clear. Moreover, in polygynous mating systems the importance of female choice remains controversial because it is often difficult to distinguish between choice for a top male *per se* and, for example, choice for a prime location that top males coincidentally prefer[6]. Perhaps more importantly, there is also the theoretical problem that strong selection for traits associated with quality will rapidly remove additive genetic variability for those traits and hence the benefits of choice, a problem termed the paradox of the lek[1–3]. Explaining why females remain choosy despite the lek paradox therefore presents a long-standing challenge to evolutionary biologists[7,8]. Here we help to resolve the paradox in fur seals by showing that female choice operates to create offspring with high heterozygosity, a trait that improves quality, but has low heritability.

Pinnipeds are interesting models for studying female choice because, although once considered classic examples of polygyny on the basis of male dominance, genetic studies have revealed disparities between behavioural and genetic estimates of male breeding success[9,10]. Even in fur seals, where agreement is better[11,12], a female may exert choice by moving away from the territory in which she spends most time. Thus, in a sympatrically breeding mixed-species colony, females become more mobile around the time of oestrus, presumably to mate with conspecific males[11].

To explore the basis of female choice in a strongly polygynous pinniped, we have studied an unusually amenable system based on Antarctic fur seals *Arctocephalus gazella* breeding at Bird Island, South Georgia. Unprecedented access is provided by an aerial walkway above the colony, allowing both extensive genetic sampling and individual positions to be determined daily to one-metre accuracy. Most if not all conceptions occur on land in the colony and unambiguous paternity can be assigned to a high proportion of pups using a panel of nine highly polymorphic microsatellites[12]. Remarkably, genetic recapture data reveal that whereas females are reasonably mobile, males rarely move more than a body-length (2 m) during the main part of the breeding season[13]. Anecdotally, we have observed individual females shifting their position within the colony around the time of oestrus, only to return subsequently to their pupping locations. Accordingly, our genetic data show that whereas 42% of females conceive to males within a radius of two body lengths (3 m, Fig. 1), appreciable numbers conceive to males up to 35 m away, suggesting that active choice is occurring. Moreover, only 23.5% of females ($n = 73$) conceive to the closest male.

Previously, we have shown that microsatellite heterozygosity is a significant predictor of male reproductive quality in terms of traits such as dominance, longevity and ability to hold territories in harsh years when pup production is low[14]. We therefore constructed a general linear model (GLM) to test the hypothesis that females that move further conceive to more heterozygous males, estimated as internal relatedness, IR[15] (see Methods), with mother-to-father relatedness fitted as an additional explanatory factor. We find a highly significant negative relationship between distance moved and male IR (Table 1; Fig. 2; $P < 0.001$), indicating that females are prepared to move further to find a more heterozygous partner. This does not appear to be an artefact of colony structure, for example females moving to where low IR males congregate, because scrambling the link between male identity and genotype completely removes

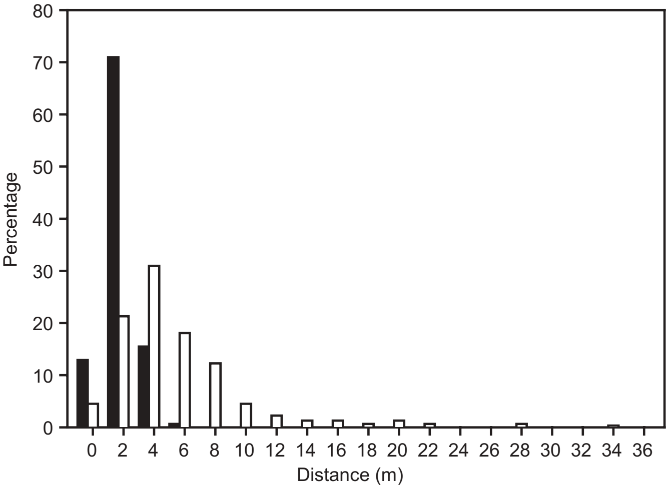

Figure 1 | Distances between male and female seals on the estimated day of conception. Black bars show the distance of each female to her nearest male, and white bars show the the distance of each female to her pup's father ($n = 310$ genetically assigned paternities).

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Table 1 | General linear model of distance moved by female, fitting father's internal relatedness (IR) and mother–father relatedness as explanatory variables

Variable	Estimate	d.f.	χ^2	P
Father's IR	−7.3357	1	7.3592	0.0008
Mother–father relatedness	−0.1710	1	2.7315	0.0672
Interaction (father's IR * mother–father relatedness)	36.6956	1	5.0572	0.0255

Data were restricted to 237 females that did not mate with their nearest male (see Methods). Total deviance = 5060.2; explained deviance = 6.6%; $n = 237$. d.f., degrees of freedom.

significance. In addition, the interaction of male IR with male–female relatedness is also significant ($P = 0.026$, Table 1), suggesting that heterozygous males are less attractive if they are closely related to the female. Again, scrambling the link between female identity and genotype removes this pattern.

Heterozygosity can impact fitness either through genome-wide patterns exposing deleterious recessive alleles (inbreeding depression) or through single locus heterosis[16]. To test for the latter, we fitted observed heterozygosity at each of the loci (as categorical variables with heterozygotes scored as one and homozygotes scored as zero) and found that every one yields a similarly non-significant but consistently positive slope (Supplementary Table 1; Sign test, $P = 0.004$). Such a pattern, with the effect spread evenly among loci rather than concentrated in one or two, suggests that the mechanism predominantly involves genome-wide (inbreeding) effects.

In many studies of polygynous species, unambiguous demonstration of female choice is difficult, even when it seems the most likely explanation of what is happening. Our system is unusual in that males, although dispersed throughout the breeding colony, remain essentially static[13]. In contrast, females tend to be more mobile and can exert choice by visiting favoured males in a way that is perhaps analogous to a dispersed lek. Movement is difficult across the crowded colony, and potentially costly both because a female has to leave her pup unattended and owing to aggression from other animals, particularly females that object to any intrusion by an adult because crushing is a major source of pup mortality[17]. Consequently, as we observe, larger movements should be made only in return for greater genetic benefits.

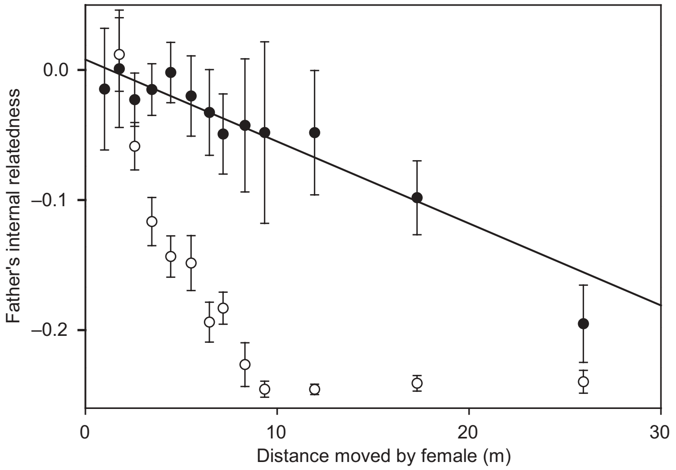

Figure 2 | Relationship between distance moved by female and father's internal relatedness (IR, filled circles). Data were restricted to 237 females that did not mate with their nearest male (see Methods). Owing to decreasing sample sizes with increasing distance moved, data are summarized (mean ± s.e.) over 1 m intervals for the first 10 metres, then over two successive 5 m intervals and finally over the remaining 15 m. The trend line (linear regression) is given for guidance only. Open circles show the mean ± s.e. minimum IR of males present within a radius of the distance moved. No data were available for the 1 m interval because the only males that were closer to the female than the father were unsampled.

We find that females choose males according to a balance between high heterozygosity and low relatedness, both of which will tend to increase offspring heterozygosity. Thus, whereas male IR predicts offspring heterozygosity (calculated as parental relatedness rather than offspring IR to maximize genetic resolution; $r^2 = 2.9\%$, $n = 310$, $P = 0.003$), female IR does not ($r^2 = 0.03\%$, $n = 310$, $P = 0.853$). In fur seals, although heterozygosity does not affect pre-weaning survival because most mortality is due to starvation and accidental crushing[17], high heterozygosity has a positive influence on many aspects of male reproductive success[14] and may well reduce disease and parasite burdens[18], indicating that females that exert choice in this way will produce fitter offspring. At the same time, our findings may help to resolve the lek paradox in two ways. First, although a few of the most heterozygous males gain many paternities, the low heritability of heterozygosity means that variability in preferred traits will not be lost. Second, because females modulate their choice according to their relatedness to potential mates, different females often favour different males. Indeed, females that move far almost invariably move more than they need simply to maximize the heterozygosity of their partner (Fig. 2). Whether additive variability is also important is uncertain, though preference for heterozygosity would tend to reduce the strength of selection on this component and hence reduce its rate of loss.

Previous solutions to the lek paradox are somewhat unsatisfactory and largely lack direct empirical support. One class of model avoids loss of additive variability by proposing low heritability for the selected trait, through either high polygenicity or environment-dependent expression[7,19]. However, low heritability implies proportionately lower benefits gained by choosy females. Similarly, choice for genetic compatibility[20] could alleviate the problem of most females choosing the same male, but has yet to be demonstrated for multilocus systems, most empirical evidence coming from the MHC[21,22]. Finally, theory indicates that selection for heterozygous partners does not solve the paradox when based on a single locus and has not been examined for multilocus scenarios[23,24]. In contrast, by choosing according to both multilocus heterozygosity and relatedness, female fur seals seem able to raise offspring quality in a way that should not exhaust additive variability for fitness.

How are females able to assess male genetic traits remotely? Heterozygosity could potentially be assessed visually through body size and/or condition[25], dominant behaviours[26], or territory quality[27]. Relatedness can be assessed olfactorily using cues from the MHC[21], but it is difficult to imagine how a female could detect genome-wide relatedness. Consequently, we refitted the GLMs, each time substituting relatedness calculated using one locus for multilocus-relatedness. Interestingly, one locus is individually significant (locus *Lw10* interaction term, $P = 0.006$), all other loci being non-significant ($P > 0.3$ in all cases). Because it is unlikely that one locus by chance lies adjacent to the MHC (if adjacent is ±5 Mb, for the human genome $P = 0.029$), our data raise the possibility that females assess relatedness using one or more MHC-like loci. As yet, we are unsure whether females are able to detect olfactory cues across a crowded colony, or instead assess males before the breeding season and remember favoured individuals through either sight or their vocalizations. Elucidating the exact mechanisms by which this is achieved should provide a fertile area for further research.

METHODS

Study site and observational data. This study was conducted at Bird Island, South Georgia (54° 00′ S, 38° 02′ W) during the austral summers of 1994/1995–2003/2004. The study population was located at a small cobblestone breeding beach covering an area of 440 m^2 and with an average pup production of 668 during the study. A scaffold walkway provided access to all parts of the beach, enabling animals to be observed and tissue-sampled with minimal disturbance.

Approximately 700 randomly selected adult females were fitted with plastic cattle ear tags (Dalton Supplies, Henley-on-Thames). Because adult territorial males were too large and aggressive to be captured, they were instead identified using distinctive natural markings (for example, scars) and by applying small patches of gloss paint to the pelage[28]. Throughout each season, daily surveys were made of all pups born to tagged females on the study beach, in which birth locations were recorded to the nearest square metre relative to grid markings painted on the scaffold walkway. We also recorded the daily locations of all adult males that held territories on the study beach.

Tissue sampling and microsatellite genotyping. Piglet ear-notching pliers were used to collect a small skin sample from the interdigital margin of the foreflipper of tagged females and their pups. Adult males were remotely sampled using a biopsy dart system. Genomic DNA was extracted and genotyped using nine dinucleotide-repeat microsatellite loci as described elsewhere[29]. The loci exhibited clear banding patterns, did not deviate significantly from Hardy–Weinberg equilibrium and were highly polymorphic, yielding up to 18 alleles per locus. The genotyping error rate, assessed by independently re-genotyping 190 individuals at all nine loci, was low at 0.0038 per reaction or 0.0022 per allele[29].

Parentage assignment, heterozygosity and relatedness. Parentage was assigned to 569 pups using a strict exclusion approach implemented in the program NEWPAT as described by Hoffman *et al.*[12]. Importantly, paternity matches were nearly identical to those assigned using a likelihood-based approach and the exclusion probability was sufficiently high (0.999964) that nearly every assignment was likely to be correct[12]. We quantified heterozygosity at nine microsatellite loci using the measure internal relatedness, IR[15]. IR estimates the relatedness of an individual's parents using the extent of allele sharing relative to random expectations by adapting the method of Queller and Goodnight[30]. Although IR is strongly correlated with standardized heterozygosity[14], it is theoretically more informative because it weights allele sharing by the frequencies of those alleles. In addition, we also calculated Queller and Goodnight's[30] relatedness between each pup's mother and father.

Statistical analyses. Spatial analyses were restricted to pups for which the mother's pupping location was known and the genetically assigned father was present on the beach within one day of the conception date ($n = 310$), estimated as the pupping day plus seven days[28]. We used the program R (http://www.r-project.org) to construct a general linear model (GLM) of distance moved by females, fitting male IR and mother–father relatedness as explanatory terms, together with first order interactions. Using standard deletion testing procedures, each term was dropped from the model unless doing so significantly reduced the amount of deviance explained. The change in deviance between the full and reduced models was distributed as χ^2 with degrees of freedom equal to the difference in the degrees of freedom between the models with and without the term in question. Because single-order terms cannot be removed from models where significant higher-order interactions are present, the significance of lower-order terms was assessed by comparison with reduced models not containing higher-order interactions. Additionally, the most parsimonious model was selected as having the lowest Akaike information criterion (AIC).

Females that mate with the nearest male may do so either because they are not choosy, or because they have exercised choice when looking where to pup and have settled close to a favoured partner. If the latter occurs, females that mate with their nearest male could include both individuals exerting no choice and those expressing strong choice, potentially confounding our analysis. Consequently the models presented use a restricted data set of 237 females that did not mate with their nearest male. However, all analyses were repeated using the full data set and yield very similar results (Supplementary Table 2). If anything, models for the full data set yield lower *P*-values (father's IR = 0.0006, mother–father relatedness = 0.0449, interaction = 0.0144) but explain less deviance (5.1%), supporting the notion that some females may pup near preferred partners.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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