

No evidence of sperm selection by female common shrews

P. STOCKLEY

Population Biology Research Group, School of Biological Sciences, Nicholson Building, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK

SUMMARY

There is currently much interest in the suggestion that females are capable of post-copulatory (or cryptic) choice for male genetic compatibility. Here, I investigate this idea using data from mixed-paternity litters of the common shrew (*Sorex araneus*). Females of this species are highly promiscuous and, in natural populations, regularly incur costs of inbreeding by mating with close relatives. Selection should therefore favour female ability for sperm selection on the basis of male relatedness. No evidence was found in support of this idea. Relative number of offspring sired within mixed paternity litters was not significantly correlated with genetic similarity of males to the female mated. Relative fertilization success was, however, significantly related to male epididymal sperm counts. I conclude that most variation in relative fertilization success of male common shrews can be explained in terms of sperm competition, and that females of this species may not be capable of sperm selection.

1. INTRODUCTION

There is currently much interest in the idea that the outcome of sperm competition (Parker 1970) in internally fertilizing species may be influenced by mechanisms of post-copulatory female choice (e.g. Eberhard 1996; Zeh & Zeh 1996, 1997). In particular, several recent studies consider the hypothesis that females (or their ova) can select sperm for fertilization on the basis of male quality or genetic compatibility (e.g. Simmons *et al.* 1996; Olsson *et al.* 1996).

Hypotheses regarding sperm selection are often difficult to test because effects of post-copulatory or 'cryptic' female choice can be difficult to distinguish from the outcome of sperm competition between males (Simmons *et al.* 1996). The most convincing evidence to date comes from genetic compatibility systems of sessile organisms in which females have no direct control over sperm transfer (see Wirtz 1997). Post-pollination female choice for genetically compatible males is well documented in a variety of flowering plants (e.g. Waser & Price 1993), and similar phenomena have been described for sessile, broadcast spawning compound ascidians with mating systems directly analogous to those of plants (Bishop 1996; Bishop *et al.* 1996).

In a recent study of sand lizards (*Lacerta agilis*), Olsson *et al.* (1996) present the first evidence to suggest that females of higher animals may also be capable of sperm selection on the basis of male genetic compatibility. Female sand lizards typically accept copulations with every male encountered, although matings with genetically similar partners lead to costs of inbreeding. Selection should therefore favour female ability to promote fertilization of their ova by genetically dissimilar males, and Olsson *et al.* (1996) show that where females

produce litters sired by males of varying relatedness to themselves, less genetically similar mating partners father proportionately more offspring. It is important that this result should be investigated further and its generality explored, because female ability for sperm selection has significant implications for understanding variance in male fertilization success, and hence determinants of reproductive success. Here, I present results of the first comparable investigation of sperm selection in a mammal, the common shrew, *Sorex araneus*. Females of this species are extremely promiscuous; in high-density populations, litters are regularly sired by up to six different males (Searle 1990; Tegelström *et al.* 1991; Stockley *et al.* 1993). Moreover, matings between genetically similar individuals such as full or half-siblings occur frequently within natural populations of the common shrew, despite associated costs of inbreeding (Stockley *et al.* 1993). The question of sperm selection in common shrews is therefore of particular interest because, like the sand lizards studied by Olsson *et al.* (1996), selection should favour female ability for post-copulatory choice based on male genetic similarity. In this paper, I examine data from naturally conceived multiply sired common shrew litters for evidence of non-random paternity in relation to genetic similarity of mating partners. I also investigate the extent to which natural variance in fertilization success is related to male sperm counts and body size, characteristics likely to reflect success in sperm competition.

2. MATERIALS AND METHODS

Common shrews are small (*ca.* 12 g) insectivorous mammals that are abundant and widely distributed throughout the northern Palaearctic region. The life cycle of the common

shrew is very simple. It is an annual species, with a breeding season in southern Britain from March to September during which females produce up to three litters of around seven offspring (Brambell 1935; Crowcroft 1957). The frequent occurrence in natural populations of matings between genetically similar individuals (Stockley *et al.* 1993) may be due to high costs associated with dispersal in this species. In general, few individuals disperse far from their natal range in the year of their birth, when it is essential to establish a territory in which to overwinter if they are to survive to breed the following spring (Shillito 1963; Michielsen 1966; Churchfield 1980).

The study population was located in a 2 ha area of undisturbed scrub-grassland adjacent to Brasenose Wood, Oxford, UK. Details of trapping methods, collection and storage of tissue samples for DNA analysis, and measurement of male body mass and epididymal sperm count are described in detail elsewhere (Stockley *et al.* 1993, 1994, 1996). Sperm counts were performed after males had been maintained in captivity for five days. This period of isolation was intended to allow for the possibility that differences in recent mating activity might influence sperm counts, although it is unlikely that much mating had occurred up to the week prior to capture, due to local synchrony in the timing of oestrus (Stockley 1996).

Paternity of offspring and parental genetic relatedness were determined using the method of multilocus DNA fingerprinting with minisatellite probes (Jeffreys *et al.* 1985; for details of protocols used and methods of scoring fingerprints, see Stockley *et al.* 1993, 1994). Paternity was assigned using two criteria: (i) when a male had all, or all but one, of the non-maternal bands of the juvenile, and (ii) when the band-share coefficient between a male and a juvenile was in the range found for mother-offspring pairs (0.37–0.72). Non-maternal band sharing coefficients were also calculated for siblings within litters to provide additional confirmation that particular individuals were full or half-siblings. Band-sharing coefficients were calculated for the parents of each juvenile to provide an index of parental genetic similarity.

Data for mixed paternity litters were standardized to give the relative proportions of offspring sired by each male, and the relative genetic similarity to the female (Olsson *et al.* 1996). Within each litter, the relative percentage of offspring sired by each male was calculated as the observed percentage of offspring sired minus the percentage expected by chance, given the number of other known sires (e.g. 50% if two males, 33% if three, etc.). The method described does not include males that had mated but failed to sire any offspring, and would not therefore reveal a discontinuous pattern of non-random paternity with respect to genetic similarity of mating partners. That is, if all males above a certain threshold level of genetic similarity with the female are prevented from achieving any fertilizations, whereas those below the same threshold are not discriminated against. However, such an effect seems unlikely in the common shrew, since offspring are regularly sired by males within the range of genetic similarity to the female of first degree relatives (Stockley *et al.* 1993). Moreover, the results of Olsson *et al.* (1996) suggest that should sperm selection occur, it is likely to vary continuously in strength with the level of genetic similarity between male and female. Exclusion from the analysis of males that had mated but failed to sire any offspring does not therefore affect the predicted relationship between known sires. The genetic similarity of each sire with the female relative to others was calculated as the male's genetic similarity to the female minus the mean genetic similarity with the female of other known sires. Litters were used as independent data points for this analysis, with each represented by one sire selected at random.

I also looked for evidence of relationships between male characteristics likely to reflect success in sperm competition

and relative fertilization success within multiply sired litters. The variables examined were cleaned body mass (as body size may determine the relative number and timing of copulations achieved) and epididymal sperm count. Individual males were used as independent data points for these analyses, with mean values of relative fertilization success calculated for those that sired offspring in more than one litter. Male common shrews adopt two discrete mating tactics that are associated with differing access to females and investment in sperm production (Stockley *et al.* 1994; 1996). Males that make long-distance movements between female ranges during the breeding season invest more in sperm production but still achieve fewer fertilizations than males with ranges consistently overlapping those of several females. Males adopting the alternative (less successful) mating tactic were therefore excluded from the analyses in relation to sperm competition.

3. RESULTS

Data were analysed for eight multiply sired common shrew litters conceived under natural conditions (Stockley *et al.* 1993). No evidence was found for a significant negative relationship between relative genetic similarity of males to their mating partner and relative fertilization success (simple least squares linear regression $F_{1,6} = 0.15$, $r^2 = 0.02$, $p > 0.70$; figure 1). There was no difference in mean genetic similarity with females mated between males adopting different mating tactics (unpaired *t*-test, $t_{20} = 1.04$, $p > 0.30$), nor was there any evidence of a significant negative relationship between relative genetic similarity of males to their mating partner and relative fertilization success when these values were calculated for males adopting the same mating tactic ($F_{1,5} = 0.27$, $r^2 = 0.05$, $p > 0.60$).

Body mass and epididymal sperm count were not significantly correlated among males included in the analyses ($F_{1,6} = 0.21$, $r^2 = 0.03$, $p > 0.65$). No significant relationship was found between male body mass and relative number of offspring fathered within multiply sired litters ($F_{1,6} = 0.002$, $r^2 = 3 \times 10^{-4}$, $p > 0.95$). There was, however, a significant positive relationship between epididymal sperm count and mean relative number of offspring sired, for males adopting the same mating tactic ($F_{1,6} = 17.04$, $r^2 = 0.74$, $p < 0.01$; figure 2).

4. DISCUSSION

The present investigation reveals no evidence of sperm selection by female common shrews on the basis of male genetic similarity. These results contrast with those of Olsson *et al.* (1996) in a recent study of sand lizards, where a strong negative association was demonstrated between male genetic similarity to the female mated and relative fertilization success. The mating pattern of common shrews parallels that of sand lizards in several respects. Most importantly, females of both species incur significant costs of inbreeding by mating with genetically similar males (Olsson *et al.* 1996; Stockley *et al.* 1993). Moreover, female shrews invest heavily in offspring during pregnancy and lactation (Genoud & Vogel 1990). The results presented here therefore suggest that female common shrews may not be capable of sperm selection on the basis of male genetic similarity, although to

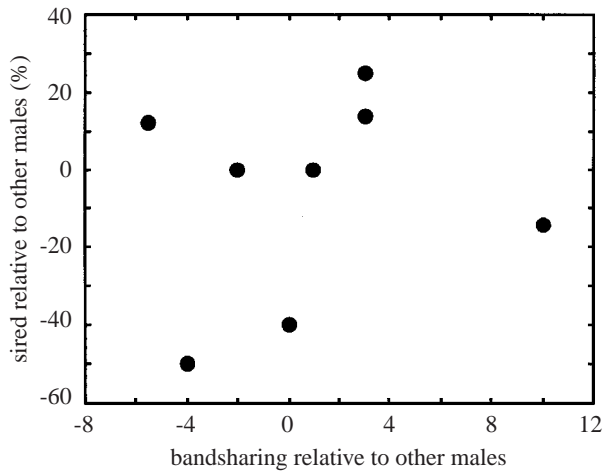


Figure 1. Relationship between relative genetic similarity of males to the female mated and relative number of offspring sired within eight multiply sired common shrew litters (simple least squares linear regression using one male per litter, $F_{1,6} = 0.15$, $r^2 = 0.02$, $p > 0.70$).

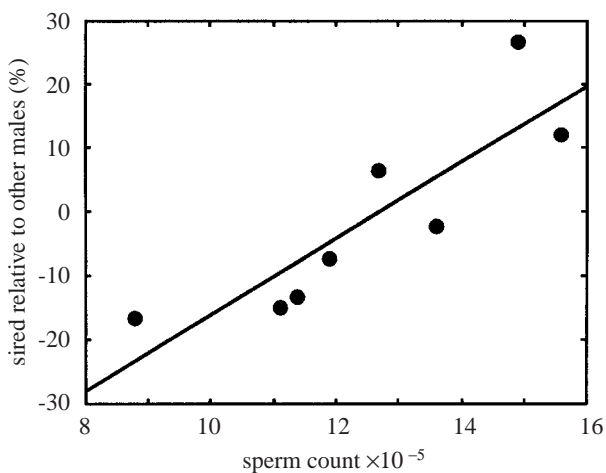


Figure 2. Relationship between epididymal sperm count $\times 10^{-5}$ and mean relative number of offspring fathered per litter for eight male common shrews with the same mating tactic (simple least squares linear regression, $F_{1,6} = 17.04$, $r^2 = 0.74$, $p < 0.01$).

demonstrate this conclusively would require an experimental approach controlling for variation in ejaculates, which is beyond the scope of the present investigation.

While the results presented here provide no evidence of sperm selection by female common shrews or their ova, it is important to note that a significant relationship between relative male fertilization success and genetic similarity with the female would not be sufficient evidence for the occurrence of such selection. Non-random paternity within litters could also be mediated by female control after fertilization, for example by selective abortion or resorption of embryos (see Birkhead & Møller 1993; Wedekind 1994). An im-

portant conclusion of the present study is that it may be unsafe to assume that females will adopt what appears the least energetically costly potential option for reducing costs of inbreeding. It is possible also that non-random paternity with respect to genetic similarity of mating partners might be generated by male effects rather than (or in addition to) female influence. There is growing evidence, for example, showing that males are able to adjust the size of their ejaculates adaptively in relation to female traits such as reproductive status, age, or body size (e.g. Wedell 1992; Shapiro *et al.* 1994; Cook & Gage 1995). Ejaculate size might therefore also be varied in relation to perceived genetic similarity with the mating partner, thereby generating patterns of non-random paternity due to sperm competition. It is important to note also that no previous study claiming to have demonstrated sperm selection has yet controlled for variation in the number or quality of sperm ejaculated (see also Simmons *et al.* 1996).

While no evidence was found in the present study for patterns of non-random paternity based on genetic similarity of mating partners, the results do suggest that much within-litter variation in paternity can be explained in terms of sperm competition between males. A significant positive relationship was found between epididymal sperm counts of males adopting a similar mating tactic and mean relative number of offspring sired within mixed paternity litters. This result is consistent with the idea of sperm competition operating along the lines of a raffle or lottery in which male fertilization success increases with the number of sperm ejaculated (Parker 1982, 1984). It also suggests that male common shrews adopting the same mating tactic achieve a similar number of copulations with each female mated, because variation between males in the number of matings achieved might otherwise override variation in ejaculate size (see also Stockley *et al.* 1996). Relative timing of copulations in relation to ovulation is also an important determinant of fertilization success for male mammals (Ginsberg & Huck 1989). However, since female common shrews are probably sexually receptive for only two hours prior to ovulation (Dehnel 1952), there may be little potential for significant variation between males in relative timing of copulations.

No significant relationship was found between the body size of male common shrews and their mean relative fertilization success within multiply sired litters. Similarly, Stockley *et al.* (1996) found no relationship between male body size and the number of females inseminated or total number of offspring sired. As suggested also by studies of agonistic encounters between common shrews in captivity (Barnard & Brown 1982; Hanski *et al.* 1991), prior residence in female home ranges may be a more important determinant of male mating success in this species than body size *per se*.

In conclusion, results of the present study do not support a hypothesis of sperm selection by female common shrews on the basis of male genetic similarity. However, much variation in relative male fertilization success is explained by variation in sperm numbers, suggesting sperm competition to be an important determinant of male reproductive success. Further studies controlling for potential effects of post-fertilization female choice,

inbreeding effects, and variation in ejaculates are needed to test the generality of these findings among mammals.

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