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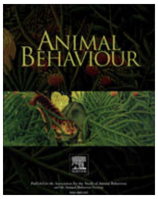

Male house mice do not adjust sperm allocation in response to odours from related or unrelated rivals

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Sperm competition theory predicts that males should adjust the number of sperm they ejaculate adaptively, according to sociosexual cues of sperm competition at the time of mating. Specifically, it is predicted that (1) males will respond to an increased risk of sperm competition from rivals by increasing sperm allocation, and (2) the increase in allocation will be lower when rivals are related. In species that use odour for communication, scent-based cues provide information on the presence and identity of conspecifics, and could thus serve as a basis for adjusting sperm allocation. We tested these predictions in the house mouse, *Mus musculus domesticus*, a species for which scent is critical to many aspects of social and reproductive communication. Sperm allocation was measured for subject males mating immediately following exposure to odour stimuli deriving from (1) themselves (control treatment), (2) a brother or (3) an unrelated male. The behavioural responses of subjects to conspecific odours indicated that males detected the presence of these stimuli in their environment, but contrary to theoretical predictions we found no evidence that they increase the number of sperm ejaculated in response to either of the odour cues indicating an elevated risk of sperm competition. Similarly, we found no significant differences between treatment groups in other traits linked to ejaculate investment, including copulatory plug size and copulatory behaviour. These findings contrast with previously studied rodent species, suggesting that responses to cues of sperm competition risk may display considerable interspecific variability.

Sperm competition occurs when the sperm of two or more males compete to fertilize a given set of ova (Parker 1970, 1998). Because male success in sperm competition often depends on the number of sperm transferred at mating (e.g. Martin et al. 1974; Preston et al. 2003), but the costs of producing large numbers of sperm are substantial (Dewsbury 1982), evolutionarily stable strategy (ESS) models have sought to predict optimal sperm allocation patterns when the likely occurrence or intensity of sperm competition varies (e.g. Parker 1990a, b, 1998, 2000; Parker et al. 1996, 1997; Parker & Ball 2005). A key prediction of such sperm competition theory is that males should increase the number of sperm transferred in a particular ejaculate in response to an elevated risk of sperm competition at the time of mating (Parker et al. 1997; Parker 1998). In an extension to this general theory, Parker (2000) considered the case in which males know their relatedness to their rivals, which affects the dynamics of sperm allocation because of the inclusive fitness benefits to be gained through the reproductive success of relatives (Hamilton 1964). Here, theory predicts that the increased sperm allocation favoured under an elevated risk of sperm competition should be reduced in direct proportion to the coefficient of relatedness of the rival (e.g. $r = 0.5$ for a brother; Parker 2000).

Consistent with the theoretical predictions outlined above (Parker et al. 1997; Parker 1998), there is now evidence that males of diverse animal taxa are able to adjust sperm allocation facultatively, and often transfer more sperm when mating under conditions associated with an elevated risk of sperm competition (reviewed in Wedell et al. 2002; see also Evans et al. 2003; Pizzari et al. 2003; delBarco-Trillo & Ferkin 2004; Pound & Gage 2004; but see Schaus & Sakaluk 2001; Ramm & Stockley 2007). The cues used by males to assess sperm competition risk vary between species, and include information relating to the presence of rival males as well as the mating status of females and of the subject male himself (see Parker et al. 1997; Wedell et al. 2002). In mammals, evidence suggests that males can adjust ejaculate allocation in response to cues including whether or not they have guarded the female prior to copulation (Norway rats, *Rattus norvegicus*: Bellis et al. 1990), the presence of a rival male (*R. norvegicus*: Pound & Gage 2004; house mice, *Mus musculus domesticus*: Preston & Stockley 2006; Ramm & Stockley 2007) and odour cues indicating the number and

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condition of rival males in the vicinity (meadow voles, *Microtus pennsylvanicus*: delBarco-Trillo & Ferkin 2004, 2006; Vaughn et al. 2008). This latter response to male odours is likely to be adaptive for many species of mammals which rely primarily on odour cues for communication (Brennan & Kendrick 2006; Roberts 2007). Moreover, since scent marks can potentially be used to assess information about individual identity and relatedness (reviewed in Thom & Hurst 2004; Brennan & Kendrick 2006), it is possible that males of some species may also use such cues to discriminate between rivals of differing relatedness when adjusting their sperm allocation strategies.

The house mouse is an ideal mammalian model to test predictions concerning sperm competition risk. Dominant male house mice aggressively defend territories against rival males, but are not always successful in monopolizing mating opportunities with resident females in their territory, since females may move between male territories and seek additional copulations (Bronson 1979). Consistent with these behavioural observations, Dean et al. (2006) reported evidence of multiple paternity in around 20% of litters in natural populations of house mice, again placing this species within the sperm competition risk range identified by Parker (1998). Male house mice use urinary scent marks to communicate with conspecific females and rival males (Hurst et al. 2001; Hurst & Beynon 2004), and so, like meadow voles (delBarco-Trillo & Ferkin 2004, 2006), may be sensitive to odour cues indicative of elevated sperm competition risk. Moreover, it is known that urinary cues encode individual identity signals in mice (Hurst et al. 2001; Cheetham et al. 2007) and are used to assess relatedness in the context of mate choice (Sherborne et al. 2007). Such cues might therefore be used as a basis for discriminating between related and unrelated rivals in sperm allocation decisions. We tested whether male house mice adjust sperm allocation in response to odour-based cues of rival males present in the environment, and also whether they vary sperm allocation decisions according to the relatedness of these rival males.

METHODS

Subjects and Husbandry

Male subjects (aged 14–19 months) were from a large, outbred colony of wild house mice maintained for six or fewer generations in captivity, originally derived from several local populations in Cheshire. Each male was individually housed under standard husbandry conditions for wild house mice, that is, in a cage measuring 48 × 11.5 cm and 12 cm high (M3, North Kent Plastic Cages Ltd., Rochester, U.K.), with Corn Cob Absorb 10/14 substrate and paper wool bedding material, and ad libitum access to food (LabDiet 5002) and water. All subjects were maintained under controlled environmental conditions (20–21 °C, relative humidity 45–65%) and a reversed 12:12 h light cycle (lights off at 0800 hours). At the end of the experiment, subject males were returned to the stock of animals maintained for use by other researchers.

Females used for each mating assay were from a pool of 44 sexually experienced BALB/c laboratory mice maintained in pairs but otherwise housed identically to males. Laboratory females were used to minimize variation caused by female effects and hence to minimize the number of animals used (Festing et al. 2002), and for their increased propensity to mate under laboratory conditions. To induce oestrus cycling prior to mating assays, females were stimulated with soiled bedding from a nonexperimental male (Marsden & Bronson 1964) and females in oestrus were identified on each morning of the experiment according to vaginal cytology (Bronson et al. 1966). If the pair failed to mate, the female was returned to the pool and used in a new mating assay at least 4 days later.

No Home Office licence or local ethical review was required for the study.

Experimental Design

We aimed to test whether male house mice adjust their investment in ejaculates in response to odour cues of an elevated sperm competition risk, and whether they respond differently to the odours of brothers and unrelated males. Odour cues were introduced to the subject males' home cages shortly before they mated, to simulate a situation whereby the subject male detects evidence of a recent intrusion into his territory, indicating an elevated risk of sperm competition with respect to the subsequent copulation. To reduce variation and minimize animal numbers, we used a matched-subject design such that each male was tested in three treatments: (1) after exposure to its own odour ('self' control treatment); (2) after exposure to the odour of an unrelated male ('unrelated male' treatment), and (3) after exposure to the odour of an unfamiliar brother ('brother' treatment). The number of sperm transferred in the first ejaculate was then compared for each male across treatments to test for evidence of differential sperm allocation in response to the presence or absence of rival male odour cues (see below). There was an interval of at least a week between treatments, and trials were balanced with respect to treatment order.

To ascertain an appropriate sample size for our study, we conducted a power analysis based on results reported by delBarco-Trillo & Ferkin (2004), which is the only previous study to have used a comparable experimental design. This study found that meadow voles adjust sperm allocation in response to odour cues from a conspecific male by increasing sperm allocation by 72% on average. Since the two treatments used by delBarco-Trillo & Ferkin (2004) are broadly equivalent to our 'self' and 'unrelated' treatment groups, we used the difference in sperm allocation from their study and estimates of between male variability from a previous study of sperm allocation in house mice (Ramm & Stockley 2007) in our power analysis, assuming that the sperm allocation in the 'brother' treatment group would be intermediate between the 'self' and 'unrelated' treatments as predicted by theory (Parker 2000). In a fully paired design, we calculated that to achieve a conventional power of 0.80 (Quinn & Keough 2002) would require a sample size of three and five pairs for the 'self'–'unrelated' and 'brother'–'unrelated' comparisons, respectively, and 0.95 power would be achieved with four and six pairs, respectively. To minimize the total number of animals used while retaining high power to detect predicted effects, we therefore designed the study to achieve at least six males mating in all three treatment groups (Festing et al. 2002).

Because male mating propensity under these experimental conditions is low (Ramm & Stockley 2007), we used a total of 22 subject males, 11 of which were excluded during the course of the experiment after failing to mate on three consecutive attempts or because they had failed to mate after two attempts by the end of the experiment. There was no evidence that the treatment group affected male mating propensity, based on whether or not each of the 22 males mated on the first occasion they were paired with a female (Fisher's exact test: $P = 0.2$). When our goal of six males mating in all three treatments had been achieved, two remaining subjects had mated in two treatments and three had mated in one treatment, giving us sperm allocation data from a total of 25 matings across 11 males. Power to detect predicted relationships of similar magnitude to those observed by delBarco-Trillo & Ferkin (2004) should therefore be high (>0.97 in both paired 'self'–'brother' and 'brother'–'unrelated' tests and an overall one-way ANOVA using data from all 25 matings). Moreover, our power to detect an effect in the opposite direction to that predicted by