Ejaculate allocation under varying sperm competition risk in the house mouse, *Mus musculus domesticus*

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A common mechanism through which males can enhance their success in postcopulatory contests over paternity is to inseminate more sperm than their rivals. However, ejaculate production is costly and the evolution of prudent sperm allocation strategies sensitive to variation in local levels of sperm competition has now been demonstrated in diverse taxa, including mammals. Theory predicts an increased sperm allocation in response to an elevated risk of sperm competition, but here we show that male house mice (*Mus musculus domesticus*) instead ejaculate fewer sperm per ejaculate when mating in the presence of a rival male. This surprising sperm allocation pattern may be a necessary consequence of adaptive changes in copulatory behavior, enabling males to achieve more rapid sperm transfer and/or to ejaculate repeatedly under risk of sexual competition. The size of a second ejaculate component, the copulatory plug, is unaffected by sperm competition risk. Our results highlight how the often complex interplay between different reproductive traits can affect the evolution of sperm competition phenotypes. **Key words:** copulatory behavior, copulatory plug, mammals, sexual selection, sperm allocation. [Behav Ecol 18:491–495 (2007)]
species experiencing higher levels of sperm competition (Ramm et al. 2005). Males might also benefit from the ability to adjust plug size intraspecifically in response to varying sperm competition risk, if, for example, a larger plug is better able to induce sperm transport (perhaps in this case compensating for the reduced physical stimulation females receive prior to ejaculation), or has a better chance of excluding or delaying the introduction of a rival male’s ejaculate.

METHODS

Subjects and housing
Our experimental males were the adult offspring of pregnant wild-caught females from Cheshire, UK, and had received prior olfactory but not physical contact with (nonexperimental) females. Experimental females were crosses of the C57BL/6 and BALB/c laboratory strains from stocks maintained at the University of Liverpool and were sexually naive. Laboratory females were used to minimize variation due to 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. Males were housed singly, and females were housed in single-sex family groups. All animals were housed in polypylene cages on Absorb 10/14 substrate with shredded paper nest material and were maintained on ad libitum food (Harlan Teklad Rodent Breeder 9607) and water. Animals were housed on a reversed phase light:dark cycle of 12:12 h (lights off at 0800 h) with all mating trials undertaken during the dark phase under low-level red lighting. Three days before mating trials, the focal males were transferred to a fresh cage, and the female selected for the experimental trial was placed in the vacated cage, as olfactory contact with male bedding can be used to induce estrus approximately 3 days later (Whitten 1957; Marsden and Bronson 1964).

Experimental protocol
Experiments were conducted in an arena measuring $58 \times 358$ cm and stimulus chamber ($19 \times 58$ cm). The screen was fitted with two 5-cm diameter double-walled wire mesh windows to permit visual and olfactory contact (but prevent physical contact) between the 2 chambers.

Each of 14 male mice was mated in 2 treatments: once in the absence and once in the presence of visual, olfactory, and auditory contact with a rival male. These treatments correspond to a “control” (i.e., that thought to occur most often under natural conditions) and “elevated” risk of sperm competition, respectively. Prior to the experiment, all males were housed singly, and so the manipulated risk of sperm competition in the 2 treatments differed only immediately prior to and during the test copulation (Engqvist and Reinhold 2005), in common with previous rodent studies (delBarco-Trillo and Ferkin 2004; Pound and Gage 2004). There was an interval of at least 1 week between mating trials to enable males time to fully recover. To control for any effect of treatment order, half the subjects were randomly assigned to mate first in the elevated sperm competition risk treatment and half in the control sperm competition risk treatment.

Before each trial, the female was moved to the mating chamber and allowed to acclimatize for 1 h. The male was placed in the vacated female’s cage to be familiarized with her odor. Ten minutes before the start of the trial, the male was moved into the mating chamber but initially confined in a Perspex tube. In the elevated sperm competition risk treatment, the rival male was added to the adjacent chamber 5 min before the start of the trial. At the start of each trial, the focal male was released and the pair continuously monitored (remotely) for mating activity. Trials were abandoned if no intromissions were observed within 90 min of their start. There was no difference between treatments in the likelihood of a trial failing (7 and 6 males failed to mate on their first attempt in the control and elevated risk treatments, respectively). Abandoned trials were repeated >1 week later.

Data collection and analysis
Successful trials were terminated 10 min after ejaculation, which is characterized by a distinctive male “shudder” and a period during which the mating pair remain immobile (Estep et al. 1975). The female was euthanized using halothane and dissected after a further 10 min. The reproductive tract was revealed by an abdominal incision, clamped at each oviduct to prevent sperm migration, and then resected rostral to the copulatory plug. The tract was opened via a longitudinal incision down each uterine horn, placed in a Perspex Sterilin tube containing 1 ml 1% citrate solution, and was agitated for 5 min to disperse the sperm. Sperm counts were performed on an Improved Neubauer hemocytometer using standard protocols (European Society of Human Reproduction and Embryology 2002). Sperm counts were validated blindly with respect to treatment by a second investigator and showed very high repeatability (Pearson correlation: $N = 26$, $r = 0.924$, $P < 0.001$). The mass of the copulatory plug was weighed immediately after dissection and after air-drying for 24 h. Copulatory behavior in male mice comprises a series of multiple preejaculatory intromissions (between which the pair separate), each comprising multiple thrusts (Estep et al. 1975). Video recordings were used to quantify the latency to first intromission and the number of intromissions and thrusts for each trial. We did not measure female copulatory behavior because previous detailed behavioral observations made using this experimental setup have shown female receptivity to be unaffected by the presence or absence of a male in the stimulus chamber (Preston and Stockley 2006). At the end of the experiment, males were euthanized and morphological data collected on body mass, testes mass, and left epididymis mass. The head and body of the right epididymis were dissected and macerated in 1 ml 1% citrate solution for 1 min and sperm counts performed as before to obtain epididymal sperm concentration.

Measurements were log or square root transformed as appropriate prior to analysis and differences between treatments assessed using paired Student’s $t$ tests. For sperm counts, we also used a repeated measures general linear model to test for any effect of treatment order. This might arise if males who first mated in the elevated sperm competition risk treatment perceived the presence of a rival to indicate an increase in the average level of sperm competition (Engqvist and Reinhold 2005).

RESULTS

A total of 13 males copulated in both treatments (one male mated in only one treatment and was therefore excluded from further analysis). There was marked variation in ejaculate size between males (ranging from $3.48 \times 10^6$ to $16.53 \times 10^6$ sperm recovered from the female reproductive tract in the control sperm competition risk treatment and from $3.41 \times 10^6$ to $14.73 \times 10^6$ in the elevated sperm competition risk treatment), but within-male ejaculate sizes across the 2 treatments were highly correlated (Pearson correlation: $N = 13$, $r = 0.88$, $P < 0.001$; Figure 1). Intermale variation in mean sperm numbers per ejaculate was uncorrelated with male body mass ($N = 13$, $r = 0.30$, $P = 0.5$), testis mass ($N = 13$, $r = 0.44$, $P = 0.1$), or
epididymis mass \( (N = 13, r = 0.36, P = 0.2) \) but was correlated with epididymal sperm concentration \( (N = 13, r = 0.64, P = 0.019) \).

Sperm numbers were found to vary consistently between treatments, with males mating in the elevated risk treatment ejaculating significantly fewer sperm than when they mated without a rival present (paired Student’s \( t \)-test: \( N = 13, t = 5.52, P < 0.001 \); Figure 2a). The mean reduction in sperm number was 29.4%. There was no significant treatment \( \times \) order interaction (repeated measures general linear model: \( F_{1,11} = 3.48, P = 0.09 \)), leaving the significant effect of treatment unaffected \( (F_{1,11} = 29.39, P < 0.001) \). There was a concomitant decline in the number of preejaculatory thrusts (paired Student’s \( t \)-test: \( N = 13, t = 3.04, P = 0.01 \)) in the elevated sperm competition risk treatment and a marginally nonsignificant trend toward fewer intromissions \( (N = 13, t = 2.11, P = 0.056) \) but no difference in intromission latency \( (N = 13, t = 1.79, P = 0.098) \), replicating previous results (Preston and Stockley 2006).

In contrast to the difference in sperm numbers, we found no difference in the size of the copulatory plug deposited by males in each of the 2 treatments (wet mass: \( N = 13, t = 0.50, P > 0.5 \); Figure 2b; dry mass: \( N = 13, t = 0.29, P > 0.5 \)), and copulatory plug masses from the same male across the 2 treatments were uncorrelated (wet mass: \( N = 13, r = 0.16, P = 0.6 \); dry mass: \( N = 13, r = 0.32, P = 0.3 \)).

**DISCUSSION**

Sperm allocation

The ability of males to manipulate the number of sperm in their ejaculate appears to be taxonomically widespread (see Introduction). However, increasing sperm allocation under a heightened risk of sperm competition (Parker 1998) can potentially be achieved through adjusting the number of sperm delivered per ejaculate, the number of ejaculates delivered to a given female, or both (Parker 1984). While recent evidence suggests that increasing ejaculation number may be an important means through which male house mice adjust their sperm allocation according to sperm competition risk (Preston and Stockley 2006), ours are the first data on per-ejaculate adjustments in sperm numbers in this species. We show that, in contrast to 2 previously studied rodent species (delBarco-Trillo and Ferkin 2004; Pound and Gage 2004), male house mice do not inseminate more sperm per ejaculate...
under a heightened risk of sperm competition. Rather, we found the opposite response: males mating under a heightened risk of sperm competition delivered fewer sperm in their first ejaculate.

While a decreased sperm allocation is predicted under certain conditions of heightened sperm competition intensity (Parker 1998), this seems unlikely to apply to our experimental setup (see Introduction; cf., delBarco-Trillo and Ferkin 2004, 2006). Rather, we suggest that this unexpected finding may be a result of concurrent changes in male copulatory behavior in the presence of a rival male. Preston and Stockley (2006) argued that premature ejaculation in the presence of a rival male reduces the risk of zero sperm transfer. Such a risk may be particularly pronounced in species such as the house mouse for which the period of copulatory behavior prior to ejaculation is very long (Estep et al. 1975; cf., Stockley and Preston 2004). Results of the present study indicate that males pay a direct cost for such premature ejaculation in terms of reduced sperm numbers in their ejaculate. The reduction in sperm numbers may result directly from the reduced stimulation males receive prior to ejaculation (Toner and Adler 1986) and is likely to reduce male competitive ability under sperm competition. This finding of Preston and Stockley (2006) that males mating in the presence of a rival were more likely to ejaculate for a second time suggests a possible compensatory mechanism for this loss of postcopulatory competitiveness, and they found that males receiving the least stimulation prior to their first ejaculation were indeed most likely to ejaculate again.

The differing response of male house mice to the presence of a rival male at the time of mating compared with previously tested rodent species (delBarco-Trillo and Ferkin 2004; Pound and Gage 2004) may relate to the divergent copulatory behavior and reproductive ecology of these species. Previously tested rodent species exhibit adaptations to a relatively high level of sperm competition, such as large testes, repeated ejaculations, and relatively short ejaculation latencies; in contrast, house mice have relatively small testes, low sperm reserves, and a low ejaculation frequency but perform prolonged bouts of copulatory stimulation prior to insemination (Huber et al. 1980; Kenagy and Trombulak 1986; Stockley and Preston 2004). Indeed, such prolonged stimulation may function in sperm competition by maximizing sperm transport (Toner et al. 1987) or reducing female receptivity to remating (Huck and Lisk 1986; see also Stockley and Preston 2004) and may also be critical to ensure female fertility (de Catanzaro 1991), suggesting a further potential cost of the males’ response in our experiment.

The mechanisms underlying the observed variation in sperm allocation dynamics among rodents are unknown, but Pound’s (1999) study of vas deferens contractility in 2 Peromyscus species with contrasting levels of sperm competition suggests one possible explanation: only the more promiscuous species in that study was found to have the receptors on the vas deferens necessary to use social stimuli indicative of sperm competition risk to alter sperm allocation (Pound 1999). Studies of vas deferens contractility in laboratory house mice have uncovered interstrain differences in responsiveness (e.g., Berti et al. 1978) but data on wild house mice are unavailable to test whether this potential mechanism is indeed relevant to our results.

Finally, as found by Pound and Gage (2004) in their study of sperm allocation in Rattus norvegicus, the numbers of sperm inseminated by individual males in the control and elevated sperm competition risk treatments were strongly correlated (see also Schaus and Sakaluk 2002; Gage and Morrow 2003 for evidence that this is a widespread phenomenon). Coupled with wide between male variation in sperm numbers, this suggests that some males may be at a consistent advantage in sperm competition.

Copulatory plug allocation

In contrast to sperm numbers, we found no evidence that male house mice respond to an elevated risk of sperm competition by adjusting the size of a second ejaculate component, the copulatory plug. If plugs function chiefly to stimulate sperm transport, males may be under strong selection to maintain plug mass: the normal size and position of the plug are critical to maximizing sperm transport in rats (Matthews and Adler 1978; Toner et al. 1987; Carballada and Esponda 1992) and the same may also be true in mice (Pang et al. 1979; Murer et al. 2001). Alternatively, if plugs function as a defensive adaptation to sperm competition (Martan and Shepherd 1976; Voss 1979; but see Dewsbury 1988), males might be predicted to increase (or at least maintain) plug size under an elevated risk of sperm competition; in some butterfly species plug efficacy increases with plug size (review in Simmons 2001), but in the primate Lemur catta the limited data available do not support a link between plug size and its retention in the female reproductive tract (Parga et al. 2006). Overall, despite an evolutionary trend toward larger plugs (and larger accessory reproductive glands, which produce the plug) in more promiscuous rodent species (Ramm et al. 2005), our understanding of the functional significance of plugs with respect to sperm competition in rodents remains incomplete (Voss 1979; Ramm et al. 2005).

Conclusions

In summary, our results suggest that there may be antagonistic pre- and postcopulatory sexual selection on the timing of ejaculation in male house mice: the modulation of copulatory behavior to perceived variation in the risk of sexual competition (Preston and Stockley 2006) can occur even at the expense of a correlated decline in initial sperm numbers, as males prioritize the insemination of a smaller ejaculate delivered with less copulatory stimulation. In some cases, males may compensate for the likely postcopulatory costs of this change in behavior by ejaculating again (Preston and Stockley 2006). Such a pattern may potentially be mediated by both behavioral and physical constraints on sperm allocation in male house mice, perhaps reflecting adaptation to a relatively low overall risk of sperm competition compared with previously studied rodent species (delBarco-Trillo and Ferkin 2004; Pound and Gage 2004; but see Dean et al. 2006 for evidence that this risk is clearly nonzero). Given previous results in a wide range of taxa (Wedell et al. 2002; Evans et al. 2003; Pizzari et al. 2003; delBarco-Trillo and Ferkin 2004; Pound and Gage 2004; but see Schaus and Sakaluk 2001), the sperm allocation dynamics reported here are somewhat surprising but underline the value of studying species with differing levels of female promiscuity and copulatory behavior in order to fully understand the diverse and often complex ways in which sperm competition shapes the evolution of male reproductive phenotypes.

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