Male house mice that have evolved with sperm competition have increased mating duration and paternity success

Ines Klemme [a],[*], Renée Claire Firman [b]

[a] Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[b] Centre for Evolutionary Biology, School of Animal Biology, The University of Western Australia, Perth, Australia

Sperm competition imposes strong selection on males to gain fertilizations and maximize paternity. Males have been shown to adapt to sperm competition by modifying their behaviour and/or reproductive physiology. We investigated the fitness effects of male responses to sperm competition in house mice, *Mus domesticus*. Males that had been evolving with (polygamy) and without (monogamy) sperm competition for 18 generations were subject to different frequencies of social encounters with conspecific males to generate a sperm competition 'risk' treatment and a 'no risk' treatment. After manipulation of their social environment for 15—22 days, males were forced to compete for fertilizations against a male with the same selection history that experienced no manipulation. We genotyped embryos at 16 days gestation to quantify paternity success, and thus the competitive ability, of the experimental males. While there was no treatment effect on mating behaviour and paternity success, males from polygamous lines had significantly increased mating duration and paternity success when positioned in the disfavoured role of the second male to mate. Thus, males that had evolved with sperm competition showed greater mating effort and fitness compared to males that had evolved without sperm competition. Whether this is due to a genetic divergence between the lines or a differential capacity of males with different selection histories to respond plastically to current levels of sperm competition remains to be tested.

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Females of most species mate with multiple males within one reproductive event, forcing the sperm of different males to compete for fertilizations (Parker 1970; Birkhead & Møller 1998; Simmons 2001). The number of sperm that each male contributes relative to the number of sperm that all other males contribute is theorized to be a key predictor of competitive fertilization success (Parker 1990). Thus, sperm competition should favour the evolution of morphological, physiological and behavioural traits that increase ejaculate expenditure. However, spermatogenesis is physiologically costly (Dewsbury 1982), and male investment in sperm production and allocation should reflect the average and current levels of sperm competition within a species and/or population.

Game theory models have been developed to predict optimal investment in relation to the probability of sperm competition and the number of competing ejaculates (i.e. the risk and intensity of sperm competition; Parker et al. 1996, 1997). To maximize their reproductive return, males are predicted to increase sperm production with increases in both average sperm competition risk and intensity (Engqvist & Reinhold 2005). However, while the allocation of existing sperm reserves should also increase with increases in the immediate risk of sperm competition, it should decrease with increases in the immediate intensity of sperm competition (Engqvist & Reinhold 2005). Consequently, males can gain fitness benefits through an awareness of the sperm competition environment, for example via cues that provide information on the number of potential rivals (e.g. density and/or sex ratio) and female mating status (Wedell et al. 2002).

Comparative analyses (e.g. Møller 1988; Gage 1994; Hosken 1997; Ramm et al. 2005; Lüpold et al. 2009) and experimental evolution studies (e.g. Hosken & Ward 2001; Pitnick et al. 2001; Simmons & Garcia-Gonzalez 2008; Firman & Simmons 2010) across many taxa have provided empirical evidence in support of the expected association between average level of sperm competition and male investment in sperm production rate, testicular tissue and accessory reproductive glands. However, only a few studies have investigated whether selection for increased ejaculate expenditure translates to fitness benefits in a competitive context (Hosken et al. 2001; Simmons & Garcia-Gonzalez 2008; Firman & Simmons 2011). In addition to evolutionary responses to sperm competition level, males have been shown to tailor their sperm expenditure according to immediate levels of sperm competition (e.g. Gage 1991; Martin & Hosken 2002; Pilastro et al. 2002; Pizzari et al. 2003; Pound & Gage 2004; delBarco-Trillo & Ferkin 2006). But only a single study has

* Correspondence: I. Klemme, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, 40014 Jyväskylä, Finland.
E-mail address: ines.klemme@jyu.fi (I. Klemme).

reported fitness effects of prudent sperm allocation: *Drosophila* males housed with rival males increased investment through mating duration and sired significantly more offspring when competing against males that were housed alone (Bretman et al. 2009).

Ejaculate components other than sperm may also be adjusted under different levels of sperm competition. Males of many species deposit a mating plug (seminal-derived coagulum) in the female reproductive tract after ejaculation (e.g. Williams-Ashman 1984). Investigations across different taxonomic groups have provided evidence to suggest that mating plugs are an evolutionary product of sperm competition (Devine 1975; Barker 1994; Dixson & Anderson 2002; Orr 2002; Ramm et al. 2005). In some species larger plugs are known to be more efficient in preventing subsequent inseminations (see Simmons 2001), while in others they may promote sperm transport (reviewed in Ramm et al. 2005). Consequently, males may benefit from adjusting the size of the plug according to the level of sperm competition.

Recently, house mice, *Mus domesticus*, have been subjected to a number of experimental studies on responses to sperm competition. Experimental evolution studies have shown genetic divergence in ejaculate quality after only eight generations; males from polygamous lines produced significantly more sperm and had better sperm motility compared to males from monogamous lines (Firman & Simmons 2010), which resulted in improved sperm competitiveness (Firman & Simmons 2011). However, experimental evidence of strategic sperm investment in response to sperm competition was shown to contradict theoretical predictions in house mice. Under risk of sperm competition, males were found to prioritize sperm transfer per se over sperm number, as they ejaculated prematurely, but repeatedly, and invested fewer sperm per ejaculation when mating in the presence of a rival (Preston & Stockley 2006; Ramm & Stockley 2007). These investigations show that male mice alter their copulatory behaviour in response to the presence of another male, but it is unclear whether males perceive this as a true risk of sperm competition. In the wild, male house mice are territorial and aggressively defend territories against rival males, and females enter male territories to engage in copulations (Bronson 1979). Thus, although female house mice are actively polyandrous (Dean et al. 2006; Firman & Simmons 2008b), it is unlikely that copulations occur in the presence of other individuals in nature.

We manipulated the perceived sperm competition 'risk' of male house mice by conspecific odorant exposure and same-sex social interactions, and then assessed whether males modulated their effort, either through an adjustment in (1) mating duration (an accurate predictor of sperm investment, Preston & Stockley 2006; Ramm & Stockley 2007) and/or (2) the size of the mating plug in response to a true risk of sperm competition by allowing these males to copulate with females that had mated previously. By assigning paternity to embryos at 16 days gestation, we were then able to quantify the fitness consequences of a perceived risk of sperm competition. Additionally, we used male house mice from experimental populations that had been evolving with (polygamous) and without (monogamous) sperm competition for 18 generations, which allowed us to assess whether selection history influenced male responses to sperm competition.

METHODS

Subjects

The mice were housed alone in standard mouse boxes (16 × 33 cm and 12 cm high) lined with aspen bedding within controlled temperature rooms maintained at 25 °C and with a reversed 14:10 h light:dark cycle. Pregnant females received shredded paper as nesting material. Rodent pellets and water were available at libitum.

The establishment and mating design of the selection lines have been described by Firman & Simmons (2010). Briefly, the ancestral population was established by randomly selecting mice from 60 litters of a colony of wild-derived house mice, which had been maintained under a monogamous mating regime for about 30 generations. Four monogamous (M) and four polygamous (P) selection lines were founded with 18 females and 18 males each. In the monogamous lines each female and each male were paired and mated with a single partner. In the polygamous lines, each female and each male were paired three times, and the same three males mated with the same three females. By randomly selecting one female and one male offspring of each family, 18 females and 18 males contributed to each generation in each replicate selection line. This selection regime minimized natural selection and allowed evolution to proceed predominantly via postcopulatory sexual selection.

We used mice from the 18th generation of three monogamous and three polygamous replicate selection lines: in total, 36 females and 54 males. The animals were approximately 24 weeks old and known to be fertile. The females had mated once and given birth to one litter before being used in this experiment.

This research was approved by the University of Western Australian Animal Ethics Committee (approval number 3/100/933).

Manipulation of Perceived Sperm Competition Risk

Male mice were housed either alone ('no risk') or with two conspecifics ('risk') in large opaque, plastic tubs (49 × 74 cm and 41 cm high). Each male was housed within a standard mouse box inside a tub. Twelve tubs were arranged in two rows in each of two 9 m² temperature-controlled rooms (Appendix Fig. A1). The distance between the tubs and the rows was standardized within each room. Each room held three 'risk' tubs (three males in each) and nine 'no risk' tubs (one male), so that there was a total of 18 'risk' males and 18 'no risk' males. In each treatment, half of the males had a monogamous selection history and half a polygamous selection history. These 36 individuals were the designated focal males. An additional 18 males (i.e. three from each of the three monogamous and three polygamous replicate selection lines) were housed individually in a third room and were designated as the nonfocal males. The home cages of these males were arranged on a bench in two staggered rows, an equal distant apart. The nonfocal males did not receive any treatments.

Focal males were subject to 22 days of manipulation of their social experience via exposure to male and female odours, and direct encounters with rival males. Male odours were introduced on days 3, 6, 10, 13, 17 and 20. 'Risk' males received alternately odours of both rival males in their tub, while 'no risk' males received only their own odour each time. Approximately 15 g of soiled bedding was taken from the front of the donor's box and introduced to the back of the recipient's box. Similarly, 15 g of soiled bedding was taken alternately from two large boxes (28 × 46 cm and 13 cm high) each housing three sexually mature females. Female odour was introduced on days 8, 15 and 22 to both 'risk' and 'no risk' males.

'Risk' males encountered both rival males in their tub on days 8, 15 and 22. For this, all three males were released one at a time, for a period of 30 min, to roam freely in the tub. Thus, males were separated by the wire-mesh bars of the box lids during the encounters, allowing visual, olfactory and acoustic contact, but no tactile contact. The order in which the three males were released