**Online supplementary material**

**Sperm sex ratio adjustment in a mammal: perceived male competition leads to elevated proportions of female-producing sperm in a mammal**

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**Methods**

**(a) Source population**

In this experiment we used third generation, lab-born wild house mice (*Mus musculus domesticus*), the ancestors of who were originally sourced from Rat Island (28 °42′S, 113 °47′E; Western Australia). Rat Island is located approximately 80km off the coast of Western Australia. The island experiences very limited marine vessel and small aircraft traffic from the mainland (i.e. there are restrictions to visiting the island). The non-endemic house mouse population residing on Rat island, which is believed to have originated from stowaways of a wrecked ship in the 1600s, is therefore a closed population [1]. On this small island (0.61 km2), house mice occur in a very small area where there is a stretch of fishing shacks (i.e. a linear distance of no more than a few hundred metres) [2]. With limited offspring dispersal capacity and the consequential overlapping of generations within the local area, it is likely that parents and offspring often experience the same social conditions. These characteristics provide good reason to expect there to be strong selection for the evolution of anticipatory parental responses within this population.

**(b) Social manipulation**

We manipulated the social experience of males during their sexual development to create variation in the perception of male-male competition risk via differential exposure to rival males [3-5]. Brothers from a total of 24 families were used across treatments in this experiment. In both treatments, males were housed in individual cages that were then placed in large plastic tubs. Thus, males were either (i) caged individually and resided alone in a large plastic tub (49 x 74 x 41cm) (‘no risk’; *n*males = 24) or (ii) caged individually and resided in a large plastic tub alongside two individually caged unrelated males (‘risk’; *n*males = 24) [3-5]. The tubs were divided evenly across two rooms so that each room contained 12 ‘no risk’ tubs and four ‘risk’ tubs. ‘Risk’ males were regularly exposed to the soiled chaff (15 g) of the two neighbouring males and once every two weeks ‘encountered’ their rivals by roaming freely within the tub (30 mins). ‘No risk’ males were regularly ‘exposed’ to their own soiled chaff, moved from the back to the front of the cage and once a fortnight released inside their tub. Although greatly reduced compared to the ‘risk’ males, the males in the ‘no risk’ treatment would have received some exposure to volatile male pheromones (i.e. due to being housed in the same room as other males). To ensure that the experimental subjects received appropriate stimuli during development, three females were (i) housed within each room, (ii) used as soiled chaff donors (15 g), and (iii) participated in male ‘encounters’ [3-5]. The exposure regimes where ceased two weeks prior to euthanasia.

**(c) Sperm isolation and DNA extraction**

Following euthanasia via cervical dislocation, the epididymides of males were removed and incised in human tubal fluid (HTF; 1mL), and incubated (37°C, 5% CO2; 10 mins) to allow the sperm to swim into the medium. After this, the tissue was removed and the suspension was incubated for longer (50 mins). Two aliquots of each sample were loaded into a haemocytometer (~10 μL) and scanned with a computer assisted sperm analyzer to measure sperm concentration. The remaining sample was centrifuged (10 mins; 14,000 rpm), washed in Tris-EDTA (500 μl), and re-centrifuged (5 mins; 14,000 rpm) to pellet the sperm. Finally, sperm were re-suspended in Tris-EDTA (200 μl) and stored (-20°C).

We extracted genomic DNA from pooled sperm samples by Chelex-100 has we have done previously [6, 7]. Briefly, a 25μl aliquot of thawed sperm solution was added to Chelex-100 resin buffer (200μl; 5%), and digested with Proteinase K (20μl; 20mg/ml) and DTT (7.6μl; 31mM) (incubation 45mins; 56°C). Following a Proteinase K enzyme deactivation period (8mins; 95°C), the samples were centrifuged (3mins; 10,500rpm) and the supernatant was quantified by a spectrophotometer. All samples were standardised to [100ng/μl] by dilution with nuclease-free water and stored (-20°C) prior to quantitative real-time polymerase chain reaction (qPCR).

**Results**

**(a) qPCR assay repeatability**

The repeatability, calculated following the method of Becker [8], of the triplicate qPCR assays was very high (*G6pd2*: R = 0.93, SE = 0.017, p << 0.0001; *Sry*: R = 0.98, SE = 0.005, p << 0.0001).

**(b) Random effects: Family ID and Tub ID**

Likelihood ratio tests (LRT) indicated that there was no significant effect of the random factor Family ID in either the testosterone LMM or the SSR GLMMs (all tests: LRT = 0, d.f. = 1, *p* = 1). The random factor Tub ID was significant in the SSR GLMMs (all tests: LRT = 75, d.f. = 1, *p* << 0.0001), but not in the testosterone LMM (LRT = 0, d.f. = 1, *p* = 1).

**References**

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