

Title	Is male reproductive senescence minimised in Mus species with high levels of sperm competition?
Authors	delBarco-Trillo, Javier;Tourmente, Maximiliano;Varea-Sánchez, María;Roldan, Eduardo R. S.
Publication date	2017-12-19
Original Citation	Delbarco-Trillo, J., Tourmente, M., Varea-Sánchez, M. and Roldan, E. R. S. (2017) 'Is male reproductive senescence minimized in Mus species with high levels of sperm competition?', Biological Journal of the Linnean Society, 123(2), pp. 463-470. doi: 10.1093/biolinnean/blx146
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://academic.oup.com/biolinnean/article-abstract/123/2/463/4761976 - 10.1093/biolinnean/blx146
Rights	© 2017 The Linnean Society of London, Biological Journal of the Linnean Society. This is a pre-copyedited, author-produced version of an article accepted for publication in Biological Journal of the Linnean Society, following peer review. The version of record is available online at: https://doi.org/10.1093/biolinnean/blx146
Download date	2026-10-07 11:53:01
Item downloaded from	https://hdl.handle.net/10468/10513



UCC

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

Is male reproductive senescence minimised in *Mus* species with high levels of sperm competition?

Javier delBarco-Trillo ^{1,2,*}, Maximiliano Tourmente ¹, María Varea-Sánchez ¹,
Eduardo R. S. Roldan ¹

¹ *Reproductive Ecology and Biology Group; Museo Nacional de Ciencias Naturales (CSIC); 28006-Madrid, Spain*

² *School of Biological, Earth and Environmental Sciences; University College Cork; Cork, Ireland*

* Corresponding author: Javier delBarco-Trillo; javier.delbarcotrillo@ucc.ie

Running title: Sperm competition and senescence

ABSTRACT

Sperm competition, an evolutionary process in which the spermatozoa of two or more males compete for the fertilisation of the same ovum, gives rise to several morphological and physiological adaptations. Generally, high levels of sperm competition enhances sperm function. In contrast, advanced age is known to lead to reproductive senescence, including a general decline in sperm function. Sperm competition and advanced age may thus have opposing effects on sperm function. Here we tested the hypothesis that the increase in sperm function in species experiencing high levels of sperm competition will counteract the negative effects of advanced age. We measured a comprehensive set of reproductive traits in young and old males in three species of mice of the genus *Mus*, which differ greatly in their levels of sperm competition. Our prediction was that the expression of reproductive senescence will be highest in the species with low levels of sperm competition and lowest in the species with high levels of sperm competition. Surprisingly, we did not find a strong signal of reproductive senescence in any of the three *Mus* species. Overall, our results did not clearly support our hypothesis that high levels of sperm competition minimise the negative effects of aging in sperm function. However, the pattern observed for the percentage of morphologically normal spermatozoa offered some support to this hypothesis.

Keywords: age and reproduction; ATP; reproductive senescence; rodents; sperm abnormalities; sperm competition; sperm function; sperm morphology

INTRODUCTION

Sperm competition occurs when a female mates with two or more males and the spermatozoa of those males compete for the fertilisation of the female's ova (Birkhead & Møller, 1998; Parker, 1970). Sperm competition is a widespread phenomenon and its occurrence leads to several evolutionary adaptations at the behavioural, morphological and physiological levels (Birkhead & Møller, 1998; delBarco-Trillo, Tourmente & Roldan, 2013). In many taxa, high levels of sperm competition are associated with an increase in the production (delBarco-Trillo *et al.*, 2013), storage and allocation of spermatozoa (delBarco-Trillo, 2011; Parker & Pizzari, 2010), as well as with enhanced sperm function (Fitzpatrick *et al.*, 2009; Gomendio *et al.*, 2006; Gómez Montoto *et al.*, 2011a; Kleven *et al.*, 2009; Martín-Coello *et al.*, 2009). For example, high levels of sperm competition in rodents lead to a higher proportion of spermatozoa that are morphologically normal, motile, and capable of reaching and fertilising the ova (Gomendio *et al.*, 2006; Gómez Montoto *et al.*, 2011a), as well as to modifications in sperm dimensions (Gomendio & Roldan, 2008; Tourmente, Gomendio & Roldan, 2011) and sperm energy metabolism (Tourmente *et al.*, 2013; Tourmente *et al.*, 2015b) that may result in improvements in sperm motility.

In contrast to the positive effect of sperm competition on sperm function, advanced age has been reported to lead to reproductive senescence (García-Palomares *et al.*, 2009a; García-Palomares *et al.*, 2009b), particularly having a negative impact on sperm function (Pizzari *et al.*, 2008). A decline in sperm function with age may be due to the accumulation of *de novo* mutations in the male germline that may occur during each cell division (Radwan, 2003), or to an increasingly impaired process of spermatogenesis with advancing age (Johnson & Gemmell, 2012; Pizzari *et al.*, 2008). These processes may be driven or exacerbated by an accumulation of reactive oxygen species and an escalation of oxidative stress with age (Johnson & Gemmell, 2012; Weir & Robaire, 2006), or by reduced efficiency of DNA repair with advancing paternal age (Paul, Nagano & Robaire, 2011; Slotter *et al.*, 2004).

Indeed, across taxa there is strong evidence for a generalised decline in sperm function with age. Such decline may involve: a decline in the number of

germinal cells and Sertoli cells in the seminiferous tubules (Dakouane *et al.*, 2005), a reduction in the number of sperm ejaculated (Sasson, Johnson & Brockmann, 2012), an increase in sperm abnormalities (Syntin & Robaire, 2001), a decrease in sperm motility (Møller *et al.*, 2009; Wolf *et al.*, 2000), an increase in sperm DNA damage (Harris *et al.*, 2011; Velando *et al.*, 2011), or decreased reproductive success (Dean *et al.*, 2010).

Although the positive effect of sperm competition on sperm function is restricted to species experiencing high levels of sperm competition, the negative impacts of advanced age on sperm function can be considered to be similar across phylogenetically related species. Consequently, we hypothesised that the generalised increase in sperm function in species with high levels of sperm competition will diminish the negative impacts of senescence only in such species. That is, in species with high levels of sperm competition, selective pressures on sperm competitiveness may be strong throughout a male's reproductive life, and may reduce the incidence of sperm senescence.

To test our hypothesis, we measured a comprehensive set of reproductive traits in young and old males in three species of mice of the genus *Mus* that differ in their levels of sperm competition based on relative testes size: *M. musculus*, *M. spretus*, and *M. spicilegus* (delBarco-Trillo *et al.*, 2016). These reproductive traits included the number of spermatozoa in the caudae epididymides, sperm dimensions and morphology, the percentage of spermatozoa with morphological abnormalities, sperm motility and velocity, and ATP content in spermatozoa. A decrease in sperm function can include lower number of stored spermatozoa, shorter spermatozoa, a higher percentage of spermatozoa with morphological abnormalities, lower motility and velocity, and lower ATP content in spermatozoa. According to our hypothesis, we predicted that the decrease in sperm function in old males would be the highest in the species with low levels of sperm competition (*M. musculus*) and the lowest in the species with high levels of sperm competition (*M. spicilegus*).

METHODS

Animals

We used adult males of three species from the genus *Mus* that differ greatly in their levels of sperm competition: *M. musculus*, *M. spretus*, and *M. spicilegus* (n = 11 per species). These three species have been characterized as a good model for studies on sperm competition in rodents, representing low, intermediate and high levels of sperm competition, respectively (Gomendio *et al.*, 2006; Gómez Montoto *et al.*, 2011a). We selected males of two age classes, hereafter referred as "young" and "old" for simplicity. Young males (n = 6 per species) were 4-6 months of age. At this age, mice are no longer juveniles but at the same time they are not old enough to be affected by reproductive senescence. Old males (n = 5 per species) were 24-28 months of age. Males were selected so that ages of young (155.11 ± 37.31 days; mean $\pm$ SD) and old animals (769.93 ± 43.96 days) were similar across species. Old males in our study were older than males considered to be senescent in other studies in mice (Anjum *et al.*, 2012; Biddle *et al.*, 1997; Tognetti *et al.*, 2017). We were not able to measure all reproductive traits for all individuals. However, $n \geq 5$ for any species and age class combination.

Adult males were close descendants of animals acquired from the Institut des Sciences de l'Evolution, CNRS- Université Montpellier 2, France, belonging to the following wild-derived strains: *M. musculus*, strain MPB (from Bialowieza, Poland); *M. spretus*, strain SEB (from Barcelona, Spain), and *M. spicilegus*, strain ZRU (from Kalomoyevka, Ukraine). Crossings in our colony were arranged to minimise inbreeding. All males were maintained under standard conditions (14 h light–10 h darkness, 22–24°C, 55-60% relative humidity); with food (rodent chow, Harlan Laboratories; seeds and fresh apple) and water provided ad libitum. All males used in this study were housed individually for at least a month before sampling to eliminate the possibility that males had a different perceived risk of sperm competition.

The research protocol was approved by the Ethics Committee of the Spanish Research Council (CSIC). All procedures were carried out following Spanish Animal Protection Regulation RD53/2013, which conforms to European Union Regulation 2010/63.

Morphological measurements

Males were sacrificed by cervical dislocation, weighed (in g) and measured (body length and tail length; in mm). To evaluate body condition we calculated a body mass index as weight (in g) / length squared (in mm²) (Labocha, Schutz & Hayes, 2014). Testes were removed and weighed (in g). Relative testes mass (RTS) has been shown to reflect sperm competition levels in rodents (Bryja *et al.*, 2008; Firman & Simmons, 2008; Long & Montgomerie, 2006; Ramm, Parker & Stockley, 2005; Soulsbury, 2010). RTS was calculated using Kenagy and Trombulak's rodent-specific regression equation: $RTS = \text{testes mass} / 0.031 \times \text{body mass}^{0.77}$ (Kenagy & Trombulak, 1986).

Compared to young mice, old mice had higher body weights (2-way ANOVA: $F_{1,27} = 23.04$, $p < 0.0001$), longer bodies ($F_{1,27} = 7.28$, $p = 0.012$), and longer tails ($F_{1,27} = 30.08$, $p < 0.0001$). Body mass index (used as a measure of body condition) was also higher in old mice than in young mice ($F_{1,27} = 8.83$, $p = 0.006$).

Relative testes size differed among the three species, following the predicted pattern with lowest values in *M. musculus* and highest in *M. spicilegus* (2-way ANOVA: $F_{2,27} = 221.75$, $p < 0.0001$; Table 1, Supporting Information). Relative testes size, however, did not differ between young and old males ($F_{1,27} = 2.51$, $p = 0.13$).

Sperm suspension preparation and sperm measurements

Mature spermatozoa were collected from the caudae epididymides and vasa deferentia, by placing the tissue in a Petri dish containing Hepes-buffered modified Tyrode's medium (mT-H; see Supporting Information for details) prewarmed to 37°C, making several cuts and allowing spermatozoa to swim out for a period of 5 min. After the 5-min swim-out incubation, the sperm suspension was transferred to a prewarmed eppendorf tube. Each sperm suspension was maintained at 37°C until processing. Some samples were assessed immediately (we will refer to this time as "0 h"). Sperm suspensions were also incubated for 3 h at 37°C in mT-H under air, after which samples were taken and some of the sperm parameters were assessed again (we will refer to this time as "3 h"). The duration of incubation (3 h) was selected based on maintenance of sperm motility in vitro in a subset of rodent species, including the three species here studied (Tourmente *et al.*, 2015b). This period of incubation does not result in a complete sperm immobilization in the

species with low sperm survival. Moreover, because fertilisation takes place a few hours after copulation in muroid species for which data are available (Suarez *et al.*, 1990), our selected incubation time is within physiological time frames.

We used a hemocytometer (modified Neubauer chamber) to estimate the total number of spermatozoa stored in the caudae epididymides. To measure sperm linear dimensions, 5 μ l of the sperm suspension was smeared onto a slide, fixed with formaldehyde in a phosphate buffer, stained with Giemsa as previously described (Gómez Montoto *et al.*, 2011a), and examined using bright field microscopy. All samples were evaluated and photographed at 1000 $\times$ magnification for subsequent digitalization using an Eclipse E-600 microscope (Nikon, Tokyo, Japan) with Pan-Fluor optics and a DS5 camera (Nikon, Tokyo, Japan). Spermatozoa were photographed by using the software NIS-Elements v.3.0 (Nikon, Tokyo, Japan). For each individual, we measured 25 different spermatozoa. Linear dimensions were obtained by measuring captured sperm images using ImageJ software v.1.41 (National Institutes of Health, Bethesda, MD, USA) (Gómez Montoto *et al.*, 2011b). Measurements included head length, head width, head area, total flagellum length, and total sperm length. Head length was measured as the linear distance between the most basal point and the most apical one of the sperm head. Head width was taken as a straight line between the dorsal and ventral regions in the wider region of the sperm head. Head area was measured considering the entire sperm head including the apical hook.

To quantify differences in sperm head morphology we used a geometric morphometric approach described previously (Varea Sánchez, Bastir & Roldan, 2013). See Supporting Information for details.

To assess sperm abnormalities, we used sperm smears stained first with eosin-nigrosin and subsequently with Giemsa (Gómez Montoto *et al.*, 2011a). Briefly, 5 μ l sperm suspension and 10 μ l eosin-nigrosin solution were mixed on a glass slide placed on a stage at 37°C and 30 s later the mix was smeared and allowed to air-dry. Smears were stained with Giemsa solution and mounted with DPX. Smears were examined at 1000 $\times$ under bright field and 200 spermatozoa per male were examined to evaluate the percentage of morphologically normal spermatozoa (i.e. without abnormal head, midpiece or principal piece, and without a cytoplasmic droplet or coiled flagella).

The percentage of motile spermatozoa (MOT) was evaluated by examining 10 μl of the sperm suspension that was placed between a pre-warmed slide and a coverslip at 100 $\times$ magnification under phase contrast optics. We also estimated the percentage of spermatozoa exhibiting forward progression. To assess sperm swimming patterns, an aliquot of sperm suspension was diluted to approximately 5×10^6 spermatozoa ml^{-1} , placed in a pre-warmed microscopy chamber with a depth of 20 μm (Leja, Nieuw-Vennep, The Netherlands), and filmed at 40 $\times$ using a phase contrast microscope connected to a digital video camera (Basler A312fc, Vision Technologies, Glen Burnie, MD). A minimum of 150 sperm trajectories were assessed per male using a computer-assisted sperm analyzer (CASA; Sperm Class Analyzer version 4.0, Microptic, Barcelona, Spain), and the following swimming parameters were estimated for each trajectory: curvilinear velocity (VCL, $\mu\text{m s}^{-1}$), straight line velocity (VSL, $\mu\text{m s}^{-1}$), average path velocity (VAP, $\mu\text{m s}^{-1}$), wobble (WOB = VAP/VCL), linearity (LIN = VSL / VCL), straightness (STR = VSL / VAP), amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF, Hz).

Sperm ATP content was measured using a luciferase-based ATP bioluminescence assay kit (HS II, Roche Applied Science) (Tourmente *et al.*, 2015a). See Supporting Information for details.

Statistical analyses

All statistical analyses were conducted using R version 3.1.0 (R Core Team, 2014) unless otherwise specified. Normality was checked with the Shapiro-Wilk normality test. If normality was not met, we used logarithmic and arcsine transformations as required. Average values are reported as mean $\pm$ SD. Significance level (α) was set at 0.05 for all the tests.

We used principal component analysis (PCA) to reduce potentially correlated variables and obtain measures of “overall sperm morphology”, “overall sperm velocity”, and “overall trajectory shape”. See Supporting Information for details.

We implemented 2-way ANOVAs and 2-way ANCOVAs fitted using the function *aov*. The two factors were species (3 levels) and age (young and old). The covariate in the ANCOVAs was body mass. We also considered the interaction between species and age to determine if any significant difference between young

and old mice differed across species, and whether such species effect paralleled the different levels of sperm competition among those species.

Geometric morphometrics statistical analyses were conducted with MorphoJ v1.06d (Klingenberg, 2011). Differences in sperm head shape between young and old individuals were quantified by examining the distance between the mean of both groups conducting a discriminant analysis (Timm, 2002).

RESULTS

The number of spermatozoa stored in the cauda epididymides differed between species, following the predicted pattern (2-way ANCOVA: $F_{2,26} = 50.36$, $p < 0.0001$; Table 1, Supporting Information), but there was no difference between young and old males ($F_{1,26} = 0.4$, $p = 0.54$).

The overall sperm morphology differed between species ($F_{2,24} = 168.42$, $p < 0.0001$), and between young and old males ($F_{1,24} = 6.15$, $p = 0.02$), but there was no significant interaction between species and age ($F_{2,24} = 0.1$, $p = 0.91$). The shape of the sperm head differed between species (discriminant analyses: $p < 0.0001$), but not between young and old mice ($p = 0.2$; Fig. 1).

The percentage of normal spermatozoa differed between species ($F_{2,27} = 7.44$, $p = 0.003$); even though there was not an overall difference between young and old males ($F_{1,27} = 0.99$, $p = 0.33$), we found a statistically significant interaction between species and age ($F_{2,27} = 4.07$, $p = 0.029$). Subanalyses by species showed no differences between young and old mice in *M. spicilegus* and *M. spretus* ($p > 0.05$) but a higher percentage of normal spermatozoa in young males than in old males in *M. musculus* ($p = 0.008$; Fig. 2), the species with the lowest level of sperm competition.

There were differences in sperm motility across species (2-way ANCOVA: $F_{2,26} = 36.47$, $p < 0.0001$), this trait being higher in young males than in old males ($F_{1,26} = 7.25$, $p = 0.01$). However, all species were affected similarly by age (interaction: $F_{2,26} = 0.1$, $p = 0.9$). After 3 hours of incubation, significant differences among species remained in sperm motility ($F_{2,26} = 25.05$, $p < 0.0001$) but there were no longer differences between age classes ($F_{1,26} = 0.41$, $p = 0.53$). Sperm forward progression also differed between species, both at 0 h ($F_{2,26} = 6.22$, $p = 0.006$) and after 3 hours of incubation ($F_{2,26} = 17.71$, $p < 0.0001$), and while it was

similar in young and old males at 0 h ($F_{1,26} = 0.25$, $p = 0.62$), after 3 hours of incubation it was higher in old males than in young males ($F_{1,26} = 7.38$, $p = 0.01$).

Overall sperm velocity and overall trajectory shape differed between species ($p < 0.0001$ in both analyses) and ages ($p < 0.01$) but there was not a significant interaction between species and age ($p > 0.05$).

There were significant differences between species in the amount of ATP per sperm cell, both at 0 h ($F_{2,26} = 6.85$, $p = 0.004$) and after 3 hours of incubation ($F_{2,26} = 9.59$, $p = 0.0008$; Table 1, Supporting Information). However, there was not a significant difference in ATP concentration between young and old males, nor a significant interaction between species and age at either time ($p > 0.05$ for all analyses).

DISCUSSION

Overall, our results do not support the hypothesis that males in species with high levels of sperm competition suffer less reproductive senescence than in species with low levels of sperm competition. We found that many reproductive traits were unaffected by age, whereas others were either enhanced or lessened in old males compared to young males, but the level of sperm competition did not have an influence in most of these traits. Only the percentage of normal spermatozoa matched our prediction, with a decline in old males in *M. musculus* (i.e. the species with low levels of sperm competition) but not in the other two species, which experience higher levels of sperm competition (*M. spretus* and *M. spicilegus*). This result may be driven by an enhanced process of spermatogenesis in species with high levels of sperm competition, which would either directly or indirectly minimise the occurrence of sperm abnormalities in old males, but this is an area of research that requires further investigation.

Even though we considered many reproductive traits that could be affected by senescence, there are many other traits that could be differently affected in young and old males of a species depending on the level of sperm competition normally experienced in that species. These traits include chromosomal abnormalities, DNA damage in spermatozoa, and any traits that regulate or determine the success of the capacitation and fertilisation processes (Gogol,

Bochenek & Smorag, 2002; Momand, Xu & Walter, 2013). For example, in brown rats, the spermatozoa of old males are more susceptible to oxidative damage and DNA fragmentation (Zubkova, Wade & Robaire, 2005), as well as having a decreased antioxidant capacity and an increased production of reactive oxygen species (Weir & Robaire, 2006). It is important to notice that the genomic damage in spermatozoa driven by aging may be independent of sperm function. Despite a normal expression of sperm function in old males, any genomic damage in their spermatozoa will increase the risk of transmission of multiple genetic and chromosomal defects to offspring (Wyrobek *et al.*, 2006).

Surprisingly, we did not find a strong signal of reproductive senescence in the three species of mice that we studied. Reproductive senescence may thus not play an important role in the natural populations of the three *Mus* species under study. Indeed, given the high predation rates suffered by rodents, most males will normally die before the inception of any signs of senescence. Another study using wild-captured *Mus musculus domesticus* found that epididymal sperm counts declined with age, although only a range of relatively advanced ages (21-32 months) were studied (Garratt *et al.*, 2011). It must be noted that most of the available knowledge on reproductive senescence in rodents is based on laboratory strains (Katz-Jaffe *et al.*, 2013; Lucio *et al.*, 2013; Parkening, 1989). This may be a shortcoming, as in a benign captive environment the negative effects of aging can be minimized and thus differences between age classes might be obscured.

Even though there are many studies describing the timing and incidence of reproductive senescence, there are also many studies in which reproductive condition remains unchanged or is even enhanced in old individuals (Gasparini *et al.*, 2010; Johnson & Gemmell, 2012; Kanuga *et al.*, 2011). It is still unclear why reproductive senescence is pronounced in some species but not in others.

It is important to consider whether any differences between young and old males lead to a fertilising advantage for one age type or the other. For example, young male guppies produce faster-swimming spermatozoa compared to old males; however, young males do not have a fertilising advantage under sperm competition scenarios (Gasparini *et al.*, 2010). It is equally possible that despite a lack of consistent differences in sperm function between young and old males, as we found in our three species of mice, undetected differences between their

spermatozoa could result in a lower reproductive potential in older individuals. We can thus conclude that even though our results did not support our hypothesis that high levels of sperm competition can minimise the impacts of senescence, more reproductive measurements, including sperm competition tests, and measurements of fertilising ability and offspring health, and possibly higher sample sizes than we used, are required to fully support or disprove our hypothesis.

ACKNOWLEDGEMENTS

This work was supported by a Ramón y Cajal fellowship from the Spanish Ministry of Economy and Competitiveness (RYC-2011-07943) and a Marie Curie Career Integration Grant (PCIG11-GA-2012-321888) to J.d.-T., a Juan de la Cierva fellowship from the Spanish Ministry of Economy and Competitiveness to M.T (JCI-2011-10381), and grants from the Spanish Ministry of Economy and Competitiveness (CGL2011-26341 and CGL2016-80577-P to E.R.S.R., and CGL2012-37423 to J.d.-T.).

We are grateful to Annie Orth and François Bonhomme (Institut des Sciences de l'Evolution de Montpellier, CNRS-Université Montpellier 2, France) for facilitating access to animals, and Juan Antonio Rielo for managing the animal facilities and Esperanza Navarro for animal care at the Museo Nacional de Ciencias Naturales (CSIC). We thank Alberto Vicens, Pilar Villar and Ester Sansegundo for their help processing spermatozoa in the laboratory. We also thank Kathryn Simmons and three anonymous reviewers for their helpful comments.

The authors have no conflicting interests to declare.

REFERENCES

Anjum S, Krishna A, Sridaran R, Tsutsui K. 2012. Localization of gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), kisspeptin and GnRH receptor and their possible roles in testicular activities from birth to senescence in mice. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* **317**: 630-644.

375 **Biddle FG, Eden SA, Rossler JS, Eales BA. 1997.** Sex and death in the mouse:
376 genetically delayed reproduction and senescence. *Genome* **40**: 229-235.

377 **Birkhead TR, Møller AP. 1998.** *Sperm Competition and Sexual Selection*. Academic
378 Press: San Diego.

379 **Bryja J, Patzenhauerová H, Albrecht T, Mošanský L, Stanko M, Stopka P. 2008.**
380 Varying levels of female promiscuity in four *Apodemus* mice species.
381 *Behavioral Ecology and Sociobiology* **63**: 251-260.

382 **Dakouane M, Bicchieray L, Bergere M, Albert M, Vialard F, Selva J. 2005.** A
383 histomorphometric and cytogenetic study of testis from men 29–102 years
384 old. *Fertility and Sterility* **83**: 923-928.

385 **Dean R, Cornwallis CK, Løvlie H, Worley K, Richardson DS, Pizzari T. 2010.**
386 Male reproductive senescence causes potential for sexual conflict over
387 mating. *Current Biology* **20**: 1192-1196.

388 **delBarco-Trillo J. 2011.** Adjustment of sperm allocation under high risk of sperm
389 competition across taxa: a meta-analysis. *Journal of Evolutionary Biology*
390 **24**: 1706-1714.

391 **delBarco-Trillo J, García-Álvarez O, Soler AJ, Tourmente M, Garde JJ, Roldan**
392 **ERS. 2016.** A cost for high levels of sperm competition in rodents:
393 increased sperm DNA fragmentation. *Proceedings of the Royal Society B:*
394 *Biological Sciences* **283**.

395 **delBarco-Trillo J, Tourmente M, Roldan ERS. 2013.** Metabolic rate limits the
396 effect of sperm competition on mammalian spermatogenesis. *Plos One* **8**:
397 e76510.

398 **Firman RC, Simmons LW. 2008.** The frequency of multiple paternity predicts
399 variation in testes size among island populations of house mice. *Journal of*
400 *Evolutionary Biology* **21**: 1524-1533.

401 **Fitzpatrick JL, Montgomerie R, Desjardins JK, Stiver KA, Kolm N, Balshine S.**
402 **2009.** Female promiscuity promotes the evolution of faster sperm in cichlid
403 fishes. *Proceedings of the National Academy of Sciences of the United States*
404 *of America* **106**: 1128-1132.

405 **García-Palomares S, Navarro S, Pertusa JF, Hermenegildo C, García-Perez MA,**
406 **Rausell F, Cano A, Tarín JJ. 2009a.** Delayed fatherhood in mice decreases

407 reproductive fitness and longevity of offspring. *Biology of Reproduction* **80**:
408 343-349.

409 **García-Palomares S, Pertusa JF, Miñarro J, García-Pérez MA, Hermenegildo C,**
410 **Rausell F, Cano A, Tarín JJ. 2009b.** Long-term effects of delayed
411 fatherhood in mice on postnatal development and behavioral traits of
412 offspring. *Biology of Reproduction* **80**: 337-342.

413 **Garratt M, Stockley P, Armstrong SD, Beynon RJ, Hurst JL. 2011.** The scent of
414 senescence: sexual signalling and female preference in house mice. *Journal*
415 *of Evolutionary Biology* **24**: 2398-2409.

416 **Gasparini C, Marino IAM, Boschetto C, Pilastro A. 2010.** Effect of male age on
417 sperm traits and sperm competition success in the guppy (*Poecilia*
418 *reticulata*). *Journal of Evolutionary Biology* **23**: 124-135.

419 **Gogol P, Bochenek M, Smorag Z. 2002.** Effect of rabbit age on sperm chromatin
420 structure. *Reproduction in Domestic Animals* **37**: 92-95.

421 **Gomendio M, Martin-Coello J, Crespo C, Magaña C, Roldan ERS. 2006.** Sperm
422 competition enhances functional capacity of mammalian spermatozoa.
423 *Proceedings of the National Academy of Sciences of the United States of*
424 *America* **103**: 15113-15117.

425 **Gomendio M, Roldan ERS. 2008.** Implications of diversity in sperm size and
426 function for sperm competition and fertility. *International Journal of*
427 *Developmental Biology* **52**: 439-447.

428 **Gómez Montoto L, Magaña C, Tourmente M, Martín-Coello J, Crespo C, Luque-**
429 **Larena JJ, Gomendio M, Roldan ERS. 2011a.** Sperm competition, sperm
430 numbers and sperm quality in muroid rodents. *Plos One* **6**: e18173.

431 **Gómez Montoto L, Varea Sánchez M, Tourmente M, Martín-Coello J, Luque-**
432 **Larena JJ, Gomendio M, Roldan ERS. 2011b.** Sperm competition
433 differentially affects swimming velocity and size of spermatozoa from
434 closely related muroid rodents: head first. *Reproduction* **142**: 819-830.

435 **Harris ID, Fronczak C, Roth L, Meacham RB. 2011.** Fertility and the aging male.
436 *Reviews in Urology* **13**: e184-e190.

437 **Johnson SL, Gemmell NJ. 2012.** Are old males still good males and can females
438 tell the difference?: Do hidden advantages of mating with old males off-set

costs related to fertility, or are we missing something else? *Bioessays* **34**: 609-619.

Kanuga MK, Benner MJ, Doble JA, Wilson-Leedy JG, Robison BD, Ingermann RL. 2011. Effect of aging on male reproduction in zebrafish (*Danio rerio*). *Journal of Experimental Zoology Part a-Ecological Genetics and Physiology* **315A**: 156-161.

Katz-Jaffe MG, Parks J, McCallie B, Schoolcraft WB. 2013. Aging sperm negatively impacts in vivo and in vitro reproduction: a longitudinal murine study. *Fertility and Sterility* **100**: 262-268.e262.

Kenagy GJ, Trombulak SC. 1986. Size and function of mammalian testes in relation to body size. *Journal of Mammalogy* **67**: 1-22.

Kleven O, Fossøy F, Laskemoen T, Robertson RJ, Rudolfson G, Lifjeld JT. 2009. Comparative evidence for the evolution of sperm swimming speed by sperm competition and female sperm storage duration in passerine birds. *Evolution* **63**: 2466-2473.

Klingenberg CP. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**: 353-357.

Labocha MK, Schutz H, Hayes JP. 2014. Which body condition index is best? *Oikos* **123**: 111-119.

Long TAF, Montgomerie R. 2006. Ejaculate investment in a promiscuous rodent, *Peromyscus maniculatus*: effects of population density and social role. *Evolutionary Ecology Research* **8**: 345-356.

Lucio RA, Tlachi-López JL, Eguibar JR, Ågmo A. 2013. Sperm count and sperm motility decrease in old rats. *Physiology and Behavior* **110**: 73-79.

Martín-Coello J, Benavent-Corai J, Roldan ERS, Gomendio M. 2009. Sperm competition promotes asymmetries in reproductive barriers between closely related species. *Evolution* **63**: 613-623.

Møller AP, Mousseau TA, Rudolfson G, Balbontín J, Marzal A, Hermosell I, De Lope F. 2009. Senescent sperm performance in old male birds. *Journal of Evolutionary Biology* **22**: 334-344.

Momand JR, Xu G, Walter CA. 2013. The paternal age effect: a multifaceted phenomenon. *Biology of Reproduction* **88**: 108.

471 **Parkening TA. 1989.** Fertilizing ability of spermatozoa from aged C57BL/6NNia
 472 mice. *Journal of Reproduction and Fertility* **87**: 727-733.

473 **Parker GA. 1970.** Sperm competition and its evolutionary consequences in
 474 insects. *Biological Reviews* **45**: 525-567.

475 **Parker GA, Pizzari T. 2010.** Sperm competition and ejaculate economics.
 476 *Biological Reviews of the Cambridge Philosophical Society* **85**: 897-934.

477 **Paul C, Nagano M, Robaire B. 2011.** Aging results in differential regulation of
 478 DNA repair pathways in pachytene spermatocytes in the brown Norway rat.
 479 *Biology of Reproduction* **85**: 1269-1278.

480 **Pizzari T, Dean R, Pacey A, Moore H, Bonsall MB. 2008.** The evolutionary
 481 ecology of pre- and post-meiotic sperm senescence. *Trends in Ecology and*
 482 *Evolution* **23**: 131-140.

483 **R Core Team. 2014.** R: A language and environment for statistical computing. R
 484 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0,
 485 URL <http://www.r-project.org/>.

486 **Radwan J. 2003.** Male age, germline mutations and the benefits of polyandry.
 487 *Ecology Letters* **6**: 581-586.

488 **Ramm SA, Parker GA, Stockley P. 2005.** Sperm competition and the evolution of
 489 male reproductive anatomy in rodents. *Proceedings of the Royal Society of*
 490 *London, Series B: Biological Sciences* **272**: 949-955.

491 **Sasson DA, Johnson SL, Brockmann HJ. 2012.** The role of age on sperm traits in
 492 the American horseshoe crab, *Limulus polyphemus*. *Animal Behaviour* **84**:
 493 975-981.

494 **Sloter E, Nath J, Eskenazi B, Wyrobek AJ. 2004.** Effects of male age on the
 495 frequencies of germinal and heritable chromosomal abnormalities in
 496 humans and rodents. *Fertility and Sterility* **81**: 925-943.

497 **Soulsbury CD. 2010.** Genetic patterns of paternity and testes size in mammals.
 498 *Plos One* **5**: e9581.

499 **Suarez S, Drost M, Redfern K, Gottlieb W. 1990.** Sperm motility in the oviduct.
 500 In: Bavister BD, Cummins J and Roldan ERS, eds. *Fertilization in Mammals*.
 501 Norwell: Serono Symposia. 111-124.

502 **Syntin P, Robaire B. 2001.** Sperm structural and motility changes during aging in
 503 the brown Norway rat. *Journal of Andrology* **22**: 235-244.

504 **Timm NH. 2002.** *Applied Multivariate Analysis*. Springer: New York.

505 **Tognetti A, Raymond M, Ganem G, Faurie C. 2017.** Females prefer cooperative
506 males even when cooperative behavior is unobserved: evidence from the
507 mound-building mouse, *Mus spicilegus*. *bioRxiv* **197988**.

508 **Tourmente M, Gomendio M, Roldan ERS. 2011.** Sperm competition and the
509 evolution of sperm design in mammals. *BMC Evolutionary Biology* **11**: 12.

510 **Tourmente M, Rowe M, González-Barroso M, Rial E, Gomendio M, Roldan ERS.**
511 **2013.** Postcopulatory sexual selection increases ATP content in rodent
512 spermatozoa. *Evolution* **67**: 1838-1846.

513 **Tourmente M, Villar-Moya P, Rial E, Roldan ER. 2015a.** Differences in ATP
514 generation via glycolysis and oxidative phosphorylation and relationships
515 with sperm motility in mouse species. *Journal of Biological Chemistry* **290**:
516 20613-20626.

517 **Tourmente M, Villar-Moya P, Varea-Sánchez M, Luque-Larena JJ, Rial E,**
518 **Roldan ERS. 2015b.** Performance of rodent spermatozoa over time Is
519 enhanced by increased ATP concentrations: The role of sperm competition.
520 *Biology of Reproduction* **93**: 64.

521 **Varea Sánchez M, Bastir M, Roldan ERS. 2013.** Geometric morphometrics of
522 rodent sperm head shape. *Plos One* **8**: e80607.

523 **Velando A, Noguera JC, Drummond H, Torres R. 2011.** Senescent males carry
524 premutagenic lesions in sperm. *Journal of Evolutionary Biology* **24**: 693-697.

525 **Weir CP, Robaire B. 2006.** Spermatozoa have decreased antioxidant enzymatic
526 capacity and increased reactive oxygen species production during aging in
527 the brown Norway rat. *Journal of Andrology* **28**: 229-240.

528 **Wolf KN, Wildt DE, Vargas A, Marinari PE, Kreeger JS, Ottinger MA, Howard**
529 **JG. 2000.** Age-dependent changes in sperm production, semen quality, and
530 testicular volume in the black-footed ferret (*Mustela nigripes*). *Biology of*
531 *Reproduction* **63**: 179-187.

532 **Wyrobek AJ, Eskenazi B, Young S, Arnheim N, Tiemann-Boege I, Jabs EW,**
533 **Glaser RL, Pearson FS, Evenson D. 2006.** Advancing age has differential
534 effects on DNA damage, chromatin integrity, gene mutations, and
535 aneuploidies in sperm. *Proceedings of the National Academy of Sciences of*
536 *the United States of America* **103**: 9601-9606.

537 **Zubkova EV, Wade M, Robaire B. 2005.** Changes in spermatozoal chromatin
538 packaging and susceptibility to oxidative challenge during aging. *Fertility*
539 *and Sterility* **84 Suppl 2:** 1191-1198.

540

542

FIGURE LEGENDS

Figure 1. Sperm head shape in young and old males of three *Mus* species. Dots indicate the landmarks used for geometric morphometrics analyses.

Figure 2. Percentage of morphologically normal spermatozoa in young and old males of three *Mus* species. For each boxplot, the bar within each box represents the sample median, each box represents 50% of the data around the median, and the two whiskers represent the 95% confidence interval. ** denotes $p < 0.001$; NS denotes $p > 0.05$.