

**UCC Library and UCC researchers have made this item openly available.
Please [let us know](#) how this has helped you. Thanks!**

Title	Is male reproductive senescence minimised in <i>Mus</i> species with high levels of sperm competition?
Author(s)	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 <i>Mus</i> species with high levels of sperm competition?', <i>Biological Journal of the Linnean Society</i> , 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 http://dx.doi.org/10.1093/biolinnean/blx146 Access to the full text of the published version may require a subscription.
Rights	© 2017 The Linnean Society of London, <i>Biological Journal of the Linnean Society</i> . This is a pre-copyedited, author-produced version of an article accepted for publication in <i>Biological Journal of the Linnean Society</i> , following peer review. The version of record is available online at: https://doi.org/10.1093/biolinnean/blx146
Item downloaded from	http://hdl.handle.net/10468/10513

Downloaded on 2021-11-27T11:29:54Z



UCC

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh

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

3

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

6

7

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

10

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

13

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

15

16 Running title: Sperm competition and senescence

17

18

19

20

21 **ABSTRACT**

22

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

41

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

44

45

46 INTRODUCTION

47

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

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

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

79 germinal cells and Sertoli cells in the seminiferous tubules (Dakouane *et al.*, 2005),
80 a reduction in the number of sperm ejaculated (Sasson, Johnson & Brockmann,
81 2012), an increase in sperm abnormalities (Syntin & Robaire, 2001), a decrease in
82 sperm motility (Møller *et al.*, 2009; Wolf *et al.*, 2000), an increase in sperm DNA
83 damage (Harris *et al.*, 2011; Velando *et al.*, 2011), or decreased reproductive
84 success (Dean *et al.*, 2010).

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

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

108

109 **METHODS**

110

111 **Animals**

112 We used adult males of three species from the genus *Mus* that differ greatly in
113 their levels of sperm competition: *M. musculus*, *M. spretus*, and *M. spicilegus* (n = 11
114 per species). These three species have been characterized as a good model for
115 studies on sperm competition in rodents, representing low, intermediate and high
116 levels of sperm competition, respectively (Gomendio *et al.*, 2006; Gómez Montoto
117 *et al.*, 2011a). We selected males of two age classes, hereafter referred as "young"
118 and "old" for simplicity. Young males (n = 6 per species) were 4-6 months of age.
119 At this age, mice are no longer juveniles but at the same time they are not old
120 enough to be affected by reproductive senescence. Old males (n = 5 per species)
121 were 24-28 months of age. Males were selected so that ages of young ($155.11 \pm$
122 37.31 days; mean \pm SD) and old animals (769.93 ± 43.96 days) were similar across
123 species. Old males in our study were older than males considered to be senescent
124 in other studies in mice (Anjum *et al.*, 2012; Biddle *et al.*, 1997; Tognetti *et al.*,
125 2017). We were not able to measure all reproductive traits for all individuals.
126 However, $n \geq 5$ for any species and age class combination.

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

138 The research protocol was approved by the Ethics Committee of the Spanish
139 Research Council (CSIC). All procedures were carried out following Spanish Animal
140 Protection Regulation RD53/2013, which conforms to European Union Regulation
141 2010/63.

142

143 **Morphological measurements**

144 Males were sacrificed by cervical dislocation, weighed (in g) and measured
145 (body length and tail length; in mm). To evaluate body condition we calculated a
146 body mass index as weight (in g) / length squared (in mm²) (Labocha, Schutz &
147 Hayes, 2014). Testes were removed and weighed (in g). Relative testes mass (RTS)
148 has been shown to reflect sperm competition levels in rodents (Bryja *et al.*, 2008;
149 Firman & Simmons, 2008; Long & Montgomerie, 2006; Ramm, Parker & Stockley,
150 2005; Soulsbury, 2010). RTS was calculated using Kenagy and Trombulak's
151 rodent-specific regression equation: $RTS = \text{testes mass} / 0.031 \times \text{body mass}^{0.77}$
152 (Kenagy & Trombulak, 1986).

153 Compared to young mice, old mice had higher body weights (2-way ANOVA:
154 $F_{1,27} = 23.04$, $p < 0.0001$), longer bodies ($F_{1,27} = 7.28$, $p = 0.012$), and longer tails
155 ($F_{1,27} = 30.08$, $p < 0.0001$). Body mass index (used as a measure of body condition)
156 was also higher in old mice than in young mice ($F_{1,27} = 8.83$, $p = 0.006$).

157 Relative testes size differed among the three species, following the predicted
158 pattern with lowest values in *M. musculus* and highest in *M. spicilegus* (2-way
159 ANOVA: $F_{2,27} = 221.75$, $p < 0.0001$; Table 1, Supporting Information). Relative
160 testes size, however, did not differ between young and old males ($F_{1,27} = 2.51$, $p =$
161 0.13).

162

163 **Sperm suspension preparation and sperm measurements**

164 Mature spermatozoa were collected from the caudae epididymides and vasa
165 deferentia, by placing the tissue in a Petri dish containing Hepes-buffered modified
166 Tyrode's medium (mT-H; see Supporting Information for details) prewarmed to
167 37°C, making several cuts and allowing spermatozoa to swim out for a period of 5
168 min. After the 5-min swim-out incubation, the sperm suspension was transferred
169 to a prewarmed eppendorf tube. Each sperm suspension was maintained at 37°C
170 until processing. Some samples were assessed immediately (we will refer to this
171 time as "0 h"). Sperm suspensions were also incubated for 3 h at 37°C in mT-H
172 under air, after which samples were taken and some of the sperm parameters were
173 assessed again (we will refer to this time as "3 h"). The duration of incubation (3 h)
174 was selected based on maintenance of sperm motility in vitro in a subset of rodent
175 species, including the three species here studied (Tourmente *et al.*, 2015b). This
176 period of incubation does not result in a complete sperm immobilization in the

177 species with low sperm survival. Moreover, because fertilisation takes place a few
178 hours after copulation in muroid species for which data are available (Suarez *et al.*,
179 1990), our selected incubation time is within physiological time frames.

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

198 To quantify differences in sperm head morphology we used a geometric
199 morphometric approach described previously (Varea Sánchez, Bastir & Roldan,
200 2013). See Supporting Information for details.

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

210 The percentage of motile spermatozoa (MOT) was evaluated by examining 10
211 μl of the sperm suspension that was placed between a pre-warmed slide and a
212 coverslip at 100 \times magnification under phase contrast optics. We also estimated the
213 percentage of spermatozoa exhibiting forward progression. To assess sperm
214 swimming patterns, an aliquot of sperm suspension was diluted to approximately
215 5×10^6 spermatozoa ml^{-1} , placed in a pre-warmed microscopy chamber with a
216 depth of 20 μm (Leja, Nieuw-Vennep, The Netherlands), and filmed at 40 \times using a
217 phase contrast microscope connected to a digital video camera (Basler A312fc,
218 Vision Technologies, Glen Burnie, MD). A minimum of 150 sperm trajectories were
219 assessed per male using a computer-assisted sperm analyzer (CASA; Sperm Class
220 Analyzer version 4.0, Microptic, Barcelona, Spain), and the following swimming
221 parameters were estimated for each trajectory: curvilinear velocity (VCL, $\mu\text{m s}^{-1}$),
222 straight line velocity (VSL, $\mu\text{m s}^{-1}$), average path velocity (VAP, $\mu\text{m s}^{-1}$), wobble
223 (WOB = VAP/VCL), linearity (LIN = VSL / VCL), straightness (STR = VSL / VAP),
224 amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF,
225 Hz).

226 Sperm ATP content was measured using a luciferase-based ATP
227 bioluminescence assay kit (HS II, Roche Applied Science) (Tourmente *et al.*,
228 2015a). See Supporting Information for details.

229

230 **Statistical analyses**

231 All statistical analyses were conducted using R version 3.1.0 (R Core Team,
232 2014) unless otherwise specified. Normality was checked with the Shapiro-Wilk
233 normality test. If normality was not met, we used logarithmic and arcsine
234 transformations as required. Average values are reported as mean \pm SD.
235 Significance level (α) was set at 0.05 for all the tests.

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

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

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

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

249

250 **RESULTS**

251 The number of spermatozoa stored in the cauda epididymides differed
252 between species, following the predicted pattern (2-way ANCOVA: $F_{2,26} = 50.36$, p
253 < 0.0001 ; Table 1, Supporting Information), but there was no difference between
254 young and old males ($F_{1,26} = 0.4$, $p = 0.54$).

255 The overall sperm morphology differed between species ($F_{2,24} = 168.42$, $p >$
256 0.0001), and between young and old males ($F_{1,24} = 6.15$, $p = 0.02$), but there was no
257 significant interaction between species and age ($F_{2,24} = 0.1$, $p = 0.91$). The shape of
258 the sperm head differed between species (discriminant analyses: $p < 0.0001$), but
259 not between young and old mice ($p = 0.2$; Fig. 1).

260 The percentage of normal spermatozoa differed between species ($F_{2,27} = 7.44$,
261 $p = 0.003$); even though there was not an overall difference between young and old
262 males ($F_{1,27} = 0.99$, $p = 0.33$), we found a statistically significant interaction
263 between species and age ($F_{2,27} = 4.07$, $p = 0.029$). Subanalyses by species showed
264 no differences between young and old mice in *M. spicilegus* and *M. spretus* ($p >$
265 0.05) but a higher percentage of normal spermatozoa in young males than in old
266 males in *M. musculus* ($p = 0.008$; Fig. 2), the species with the lowest level of sperm
267 competition.

268 There were differences in sperm motility across species (2-way ANCOVA:
269 $F_{2,26} = 36.47$, $p < 0.0001$), this trait being higher in young males than in old males
270 ($F_{1,26} = 7.25$, $p = 0.01$). However, all species were affected similarly by age
271 (interaction: $F_{2,26} = 0.1$, $p = 0.9$). After 3 hours of incubation, significant differences
272 among species remained in sperm motility ($F_{2,26} = 25.05$, $p < 0.0001$) but there
273 were no longer differences between age classes ($F_{1,26} = 0.41$, $p = 0.53$). Sperm
274 forward progression also differed between species, both at 0 h ($F_{2,26} = 6.22$, $p =$
275 0.006) and after 3 hours of incubation ($F_{2,26} = 17.71$, $p < 0.0001$), and while it was

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

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

281 There were significant differences between species in the amount of ATP per
282 sperm cell, both at 0 h ($F_{2,26} = 6.85$, $p = 0.004$) and after 3 hours of incubation ($F_{2,26}$
283 $= 9.59$, $p = 0.0008$; Table 1, Supporting Information). However, there was not a
284 significant difference in ATP concentration between young and old males, nor a
285 significant interaction between species and age at either time ($p > 0.05$ for all
286 analyses).

287

288 **DISCUSSION**

289

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

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

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

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

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

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

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

349

350 **ACKNOWLEDGEMENTS**

351

352 This work was supported by a Ramón y Cajal fellowship from the Spanish
353 Ministry of Economy and Competitiveness (RYC-2011-07943) and a Marie Curie
354 Career Integration Grant (PCIG11-GA-2012-321888) to J.d.-T., a Juan de la Cierva
355 fellowship from the Spanish Ministry of Economy and Competitiveness to M.T (JCI-
356 2011-10381), and grants from the Spanish Ministry of Economy and
357 Competitiveness (CGL2011-26341 and CGL2016-80577-P to E.R.S.R., and
358 CGL2012-37423 to J.d.-T.).

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

366 The authors have no conflicting interests to declare.

367

368 **REFERENCES**

369

370 **Anjum S, Krishna A, Sridaran R, Tsutsui K. 2012.** Localization of gonadotropin-
371 releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH),
372 kisspeptin and GnRH receptor and their possible roles in testicular
373 activities from birth to senescence in mice. *Journal of Experimental Zoology*
374 *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

439 costs related to fertility, or are we missing something else? *Bioessays* **34**:
440 609-619.

441 **Kanuga MK, Benner MJ, Doble JA, Wilson-Leedy JG, Robison BD, Ingermann**
442 **RL. 2011.** Effect of aging on male reproduction in zebrafish (*Danio rerio*).
443 *Journal of Experimental Zoology Part a-Ecological Genetics and Physiology*
444 **315A**: 156-161.

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

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

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

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

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

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

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

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

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

469 **Momand JR, Xu G, Walter CA. 2013.** The paternal age effect: a multifaceted
470 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

543 **FIGURE LEGENDS**

544

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

547

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

553