EVOLUTIONARY CHANGE IN TESTES TISSUE COMPOSITION AMONG EXPERIMENTAL POPULATIONS OF HOUSE MICE

Journal:	<i>Evolution</i>
Manuscript ID:	Draft
Manuscript Type:	Brief Communication
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Firman, Renee; University of Western Australia, Animal Biology (M092); Garcia-Gonzalez, Francisco; University of Western Australia, Centre for Evolutionary Biology Tyler, Evan; University of Western Australia, Centre for Evolutionary Biology Wheeler, Samantha; University of Western Australia, Centre for Evolutionary Biology Yamin, Zayaputeri; University of Western Australia, Centre for Evolutionary Biology Yuan, Michael; University of Western Australia, Centre for Evolutionary Biology Simmons, Leigh; University of Western Australia, Centre for Evolutionary Biology; University of Western Australia, School of Animal Biology (M092)</p>
Keywords:	Mating Systems, Selection - Experimental, Selection - Sexual

BRIEF COMMUNICATION

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

**EVOLUTIONARY CHANGE IN TESTES TISSUE
COMPOSITION AMONG EXPERIMENTAL
POPULATIONS OF HOUSE MICE**

**Renée C. Firman^{1,2}, Francisco Garcia-Gonzalez^{1,3}, Evan Thyer¹,
Samantha Wheeler¹, Zayaputeri Yamin¹, Michael Yuan¹ and
Leigh W. Simmons¹**

*¹Centre for Evolutionary Biology, School of Animal Biology (M092),
University of Western Australia, Nedlands 6009, Australia*

²E-mail: renee.firman@uwa.edu.au

³Estacion Biologica de Doñana-CSIC, 41092, Sevilla, España

Running head: Evolutionary change in the testes in mice

23 Theory assumes that postcopulatory sexual selection favours increased investment in
24 testes size because greater numbers of sperm within the ejaculate increase the chance
25 of success in sperm competition, and larger testes are able to produce more sperm.
26 However, changes in the organization of the testes tissue may also affect sperm
27 production rates. Indeed, recent comparative analyses suggest that sperm competition
28 selects for greater proportions of sperm-producing tissue within the testes. Here, we
29 explicitly test this hypothesis using the powerful technique of experimental evolution.
30 We allowed house mice (*Mus domesticus*) to evolve via monogamy or polygamy in
31 six replicate populations across 24 generations. We then used histology and image
32 analysis to quantify the proportion of sperm-producing tissue (seminiferous tubules)
33 within the testes of males. Our results show that males that had evolved with sperm
34 competition had testes with a higher proportion of seminiferous tubules compared
35 with males that had evolved under monogamy. Previously, it had been shown that
36 males from the polygamous populations produced greater numbers of sperm in the
37 absence of changes in testes size. We thus provide unequivocal evidence that sperm
38 competition selects for an increase in the density of sperm-producing tissue, and
39 consequently increased testes efficiency.

40

41

42

43

44

45 **KEY WORDS:** Sperm competition, postcopulatory sexual selection, sperm
46 production, testosterone production, histology

47

48 Parker's game theoretic models of ejaculate evolution assume that sperm competition
 49 conforms to a raffle, with the relative number of sperm from each competitor being
 50 the primary determinant of paternity success (Parker and Pizzari 2010). In support of
 51 sperm competition theory, evolutionary and reproductive biologists focusing on *in*
 52 *vivo* sperm competition dynamics have shown that males that produce ejaculates with
 53 high numbers of sperm typically have a fertilization advantage over their rival/s (e.g.
 54 domestic species, Beatty 1957; Martin et al. 1974; wild species, Gage and Morrow
 55 2003; Boschetto et al. 2011; Firman and Simmons 2011). Thus, when male fitness is
 56 contingent on the number of sperm within the ejaculate, it is likely that postcopulatory
 57 selection will target the organ responsible for sperm production, and evolutionary
 58 change will ensue (Parker 1970).

59 A positive correlation between testes size and the level of sperm competition, as
 60 determined by variation in mating system, the frequency of female remating,
 61 differences in population sex ratio, etc, has been observed. A multitude of studies
 62 have implicated intense selection via sperm competition as favoring the evolution of
 63 larger testes both within (Ribble and Millar 1992; Brown and Brown 2003; Long and
 64 Montgomerie 2005; Firman and Simmons 2008; Dzminiski et al. 2009) and between
 65 species (e.g. primates, Harcourt et al. 1981; butterflies, Gage 1994; bats, Hosken
 66 1997; frogs, Byrne et al. 2002; birds, Pitcher et al. 2005; scorpions, Vrech et al. 2014).
 67 To date however, the most convincing evidence for the role of sperm competition in
 68 testes size evolution comes from investigations that have maintained evolving
 69 laboratory populations, and found reductions in testes mass with the removal of sperm
 70 competition (Hosken and Ward 2001; Pitnick et al. 2001; Simmons and Garcia-
 71 Gonzalez 2008). This significant body of literature has resulted in testes size (relative
 72 to body size) being routinely used as an indirect measure of sperm competition risk.

73 Unfortunately, the underlying critical assumption that testes size accurately reflects
74 sperm production rate has largely been overlooked (discussed by Schärer et al. 2004).
75 Certainly, variation in testis size alone may not necessarily reflect differences in
76 sperm production rate, and evidence has emerged to support the idea that sperm
77 competition selects to maximise testicular efficiency beyond changes in testes size
78 (reviewed in Ramm and Schärer 2014).

79 The testis consists of spermatogenic tissue, which includes germ cells and
80 somatic cells (called Sertoli cells in mammals), and non-spermatogenic tissue with
81 somatic cells only (e.g. blood vessels and Leydig cells). In higher vertebrates the germ
82 cells are located within the long and convoluted seminiferous tubules (Gier and
83 Marion 1970; Roosen-Runge 1977; Wistuba et al. 2007). During the process of
84 spermatogenesis the germ cells develop from spermatogonia into spermatocytes, then
85 to spermatids, and finally spermatozoa (Roosen-Runge 1977; Wistuba et al. 2007).
86 Spermatozoa are released from the nourishing Sertoli cells to enter the lumen of the
87 seminiferous tubule before being passed into the efferent ducts and transported to the
88 epididymis for maturation. The interstitial tissue that surrounds the seminiferous
89 tubules supplies blood to the testis and produces endocrine signals via the Leydig
90 cells (Wistuba et al. 2007).

91 It has long been known that seasonal variation in the amount of sperm-
92 producing tissue versus non sperm-producing tissue occurs in many different taxa
93 (e.g. Mayhew and Wright 1970; Goldberg and Parker 1975; Hochereau-de Reviers
94 and Lincoln 1978; Fuentes et al. 1991). Since Harcourt *et al.* (1981) first indicated
95 that sperm competition has the potential to select for increased sperm production rates
96 via alternations in the spatial organization of the testes, a number of comparative
97 studies have explored the association between the proportion of sperm-producing

98 tissue within the testes and postcopulatory sexual selection (Wistuba et al. 2003;
 99 Lüpold et al. 2009; Rowe and Pruett-Jones 2011; Montoto et al. 2012). Interspecies
 100 comparisons have provided general evolutionary patterns, but fail to demonstrate
 101 cause and effect. In their review on the evolutionary ecology of testicular function,
 102 Ramm and Schärer (2014) called for researchers to explore directly the consequences
 103 of sperm competition for testes machinery by using the powerful technique of
 104 experimental evolution. Here we provide the first account. We quantified the amount
 105 of sperm-producing and interstitial tissue from images of histological preparations of
 106 the testes of male house mice (*Mus domesticus*) from six replicate populations that
 107 had been evolving under a monogamous or polygamous mating regime for 24
 108 generations. Our analyses revealed that the testes of males that had evolved with
 109 sperm competition had greater proportions of seminiferous tubules compared to males
 110 that had evolved via monogamy. Previously, it had been shown that males from the
 111 polygamous populations produced ejaculates with elevated sperm numbers in the
 112 absence of changes in testes size (Firman and Simmons 2010). Thus, our result here
 113 suggests that sperm competition acts directly on the spatial organisation of the testes,
 114 which is likely to select for enhanced efficiency and the production of more sperm.

115

116 *Materials and Methods*

117 **EXPERIMENTAL ANIMALS**

118 House mice are actively polygamous in nature, with both males and females
 119 copulating with multiple mates (Dean et al. 2006; Firman and Simmons 2008).
 120 However, laboratory strains of house mice are typically maintained under an enforced
 121 monogamous mating regime. The Uwa:MMD colony of wild-derived mice was
 122 established in 1997 at the Animal Resources Centre (ARC) (Murdoch, Western

123 Australia) with 90 breeding pairs sourced from six wild, Australian *Mus domesticus*
 124 populations. From 1997 to 2002 the colony was maintained as an outbred population
 125 under the Poiley outbreeding system (Poiley 1960), after which the outbreeding
 126 regime changed to a dedicated program based on the coefficient of inbreeding
 127 (Firman and Simmons 2010). Consequently, the Uwa:MMD colony was maintained
 128 under a strict monogamous mating regime for approximately 30 generations prior to
 129 the establishment of the selection lines. Male/female pairs ($n = 60$) reproduced to
 130 establish a base population from which our selection lines were derived, for which we
 131 referred to the ARC colony pedigree to ensure that close relatives did not breed.

132 At the University of Western Australia the animals were maintained in a
 133 constant temperature room under identical conditions (24°C; reversed 14:10 hour
 134 light-dark cycle), and food and water was provided *ad libitum*. We established four
 135 monogamous and four polygamous lines with 18 males and 18 females in each.
 136 Subsequently, 18 males and 18 females contributed to each generation. In the
 137 monogamous lines, almost all selection on adult fitness was eliminated by ensuring
 138 that every male and female pair contributed one son and one daughter to the next
 139 generation (Shabalina et al. 1997). Although greatly relaxed, selection may not have
 140 been completely eliminated because sometimes a pair did not mate or produce
 141 offspring. In the polygamous lines, adult females had equal fitness (two offspring),
 142 and adult males had equal mating success but not equal fertilisation success due to
 143 sperm competition and/or cryptic female choice. Four polygamous lines were
 144 established with 18 females and 18 males, but potentially <18 sires. In the
 145 polygamous lines, the same three males mated with the same three females. Thus,
 146 males in the polygamous lines competed for fertilizations, and the number of males
 147 who contributed to successive generations was determined by the relative paternity

148 success of each male. This mating design ensured that the effective population sizes
149 of the monogamous lines were potentially greater than the polygamous lines at each
150 generation. Therefore, any observed fitness benefits associated with sperm
151 competition would be conservative, as the polygamous lines would be expected to
152 have higher inbreeding coefficients than the monogamous lines (documented at
153 generation 12; Firman & Simmons 2011). As with the monogamous lines, one male
154 and one female were selected at random from each polygamous line litter and used to
155 produce the next generation. Consequently, while postcopulatory sexual selection on
156 males was reinstated in the polygamous lines, natural selection and precopulatory
157 sexual selection remained absent in both treatments.

158 Anatomical measurements and sperm quality assays initially performed after
159 eight generations of experimental evolution revealed that males had diverged in sperm
160 number without changes in testes size (Firman and Simmons 2010; Table S1 in the
161 online supplementary material). Males from the polygamous lines produced more
162 sperm, had a greater proportion of motile sperm, and sperm with greater swimming
163 velocities than males from the monogamous lines, as would be expected following
164 selection via sperm competition (Firman and Simmons 2010). Further, testes size
165 among males from the different selection regimes had not changed following 16
166 (Firman et al. 2011) or 18 (Table S1; Table S2) generations of selection. Males from
167 the 24th generation were used here to assess evolutionary change in the spatial
168 organisation of the testes. Due to time and resource restrictions, we were limited to
169 using a randomly selected sample of three monogamous (n = 10 males/line) and three
170 polygamous (n = 10 males/line) selection lines.

171 Variation in the social environment can induce plasticity in sperm production in
172 house mice (Ramm and Stockley 2009; Firman et al. 2013). Consequently, the

173 animals used in this experiment were reared under identical conditions. Thus, after
174 being weaned from their mother at three weeks of age, each male was housed in an
175 individual cage until they reached sexual maturity (12 weeks of age). To ensure that
176 males acquired the appropriate olfactory cues during their sexual development they
177 were placed within close proximity to cages housing females. All males were virgins
178 at the time of sacrifice.

179

180 **TESTES HISTOLOGY AND IMAGE ANALYSIS**

181 Males were sacrificed via lethal injection and stored at -20°C. Prior to dissection, the
182 bodies were defrosted and weighed. A single testis from each male was chosen at
183 random and fixed in 10% buffered formal saline. Each testis was dehydrated through
184 a series of graded ethanol baths, chloroform baths, and paraffin, and then embedded in
185 paraffin wax. The specimens were sectioned through the midline, and centre sections
186 of the testis were mounted on slides. The slides were stained with Gill's haematoxylin
187 and Eosin and viewed under a BX50 (Olympus) light microscope (×10 objective).
188 Five different images per testis were captured using an Olympus DP72 camera
189 attached to the microscope. Testis tissue covered the entire area in each image (Fig.
190 2).

191 Each image was visualized using the image analysis software ImageJ. We
192 quantified the proportion of sperm-producing tissue within each image. Thus, we
193 measured and recorded the area of each seminiferous tubule (including the tubule
194 lumen) within each image (see Fig. S1 in the online supplementary material). We then
195 calculated the proportion of sperm-producing tissue per image: $\text{sum}[\text{seminiferous tubule area}]/\text{total image area}$. The 300 images were distributed randomly among four
197 different investigators, and each investigator was blind to the treatment from which

198 the images were sourced.

199

200 **STATISTICAL ANALYSES**

201 To confirm that investigator bias did not influence our measurements and results, a set
202 of eight images were measured by the four investigators, which allowed us to perform
203 a repeatability analysis on those measurements. A Levene's Test was applied to test
204 the equality of variances in the proportion of sperm-producing tissue among the
205 replicate lines (*SciStat Calc* 2013). We used JMP statistical software to perform a
206 nested analysis of variance (ANOVA) with replicate lines nested within selection
207 treatments (random effect) to account for non-independence of replicate lines. Effect
208 size analysis was calculated using the library "compute.es" in R. All means are
209 presented ± 1 SE.

210

211 **Results**

212 **SPERM NUMBER AND TESTES SIZE MEASUREMENTS**

213 The repeatability analysis revealed that there was no measurement bias among the
214 investigators. There was significantly more variation between images than within
215 images ($F_{7,24} = 17.171$, $P < 0.001$; $R = 0.500$, calculated following Becker 1984).
216 We calculated the mean proportion of sperm-producing tissue for each male. A
217 Levene's Test revealed that the variances in the mean proportion of sperm-producing
218 tissue were equal among the replicate lines ($n = 6$, $W = 0.778$, $P = 0.570$). Thus, we
219 looked for an effect of selection history among males from the different experimental
220 populations.

221 A nested ANOVA revealed significant variation in the proportion of total tubule
222 area among males from the polygamous and monogamous selection lines (Table 1).

223 The mean difference in the proportion of sperm-producing tissue between treatments
 224 was 4.6%. Males from the polygamous lines had testes with greater proportions of
 225 sperm-producing tissue ($n = 3$, 0.747 ± 0.008) compared with males from the
 226 monogamous lines ($n = 3$, 0.701 ± 0.009) (Fig. 1; Fig. 2). We calculated a
 227 standardized mean difference effect size (Cohen's d) and the associated 95%
 228 confidence intervals (CIs) to gauge the magnitude of the observed effect of selection
 229 history ($d = 4.66$ [0.29, 9.03]) on the proportion of total tubule area. The effect size is
 230 large, and the 95% CIs give us confidence in rejecting the hypothesis that selection
 231 history had no effect on tubule area.

232

233 *Discussion*

234 Testes size evolution via postcopulatory sexual selection is well evidenced
 235 (Gomendio et al. 1998; Simmons 2001; Birkhead et al. 2009). Both inter- and intra-
 236 species comparisons have shown that sperm competition typically favours an increase
 237 in the amount of spermatogenic tissue, and thus larger testes (e.g. Gage 1994;
 238 Hosken 1997; Byrne et al. 2002; Firman and Simmons 2008; Dzminiski et al. 2009).
 239 However, in addition to gross testes size, the relative proportion of sperm-producing
 240 tissue within the testes could also be an important factor determining spermatogenic
 241 investment (Ramm and Schärer 2014). Certainly, the capacity of the mammalian
 242 testes to produce sperm varies considerably across taxa; among six rodent species the
 243 proportion of sperm-producing tissue within the testes has been shown to range from
 244 33% to 90% (Russell et al. 1990). Within *Mus*, the level of sperm competition (as
 245 estimated by relative testes size) correlates with both the number of sperm produced
 246 (Montoto et al. 2011) and the proportion of seminiferous tubules within the testes
 247 (Montoto et al. 2012). Similar evolutionary associations between the intensity of

248 sperm competition and testes tissue organization have been observed in comparative
249 studies of two groups of birds, the New World blackbirds (Icteridae) and the
250 Australian passerines (Maluridae) (Lüpold et al. 2009; Rowe and Pruett-Jones 2011).
251 Here, we show that male house mice from experimental populations evolving with
252 sperm competition have testes with greater proportions of sperm-producing tissue
253 compared to the testes of males from populations maintained under enforced
254 monogamy.

255 An increase in the proportion of spermatogenic tissue in the testes of males from
256 the polygamous lines could be ascribed to selection acting on standing genetic
257 variation in testes tissue organisation. Alternatively, as we reinstated sperm
258 competition in a source population that had experienced a long history of monogamy,
259 in which deleterious mutations might have accumulated, our result could be explained
260 by sperm competition successfully purging mutations that influenced testes
261 phenotype. While theory predicts that sexual selection on males can substantially
262 reduce the accumulation of mildly deleterious mutations (McLearn and Manning
263 1985; Whitlock and Agrawal 2009), experimental evidence among different
264 invertebrate species is contradictory (e.g. Radwan 2004; Hollis et al. 2009; Arbuthnott
265 and Rundle 2012; Almbro and Simmons 2014). To date, there have been no direct
266 tests of whether mutation accumulation influences testes tissue composition, however
267 it is interesting to note that a recent study of the dung beetle *Onthophagus taurus*
268 found that sexual selection was ineffective in removing deleterious mutations that
269 reduced testes mass (Almbro and Simmons 2014).

270 Deleterious recessive mutations can be exposed under conditions of inbreeding
271 (Charlesworth and Charlesworth 1999). Consequently, if individuals from our
272 monogamous selection lines were ‘carrying’ high mutation loads they might be

273 expected to experience greater fitness consequences from reproducing with relatives
274 compared with individuals from the polygamous lines. Within-line experimental
275 matings performed after 16 generations of selection revealed that full-sibling parental
276 pairs and unrelated parental pairs had equivalent fitness (Firman *et al.* 2011). Further,
277 the resulting ‘inbred’ polygamous and ‘inbred’ monogamous male offspring had, on
278 average, comparable testes sizes and sperm numbers (Firman *et al.* 2011). Thus,
279 although we are unable to eliminate conclusively that sperm competition purged
280 deleterious alleles that influenced testes phenotype and suppressed testes function,
281 available data suggests that this was not the case. Regardless, we have provided
282 compelling evidence that sperm competition selects for enhanced levels of sperm-
283 producing tissue, either by selecting against males with high mutation loads, or by
284 acting on standing genetic variation. Certainly, these two mechanisms are not
285 mutually exclusive and are likely to co-occur in both experimental and natural
286 populations.

287 A series of previous investigations performed using these lineages of house
288 mice had revealed that males from populations evolving via sperm competition
289 produced more sperm compared with males from populations evolving under
290 monogamy, but that changes in sperm production were not associated with changes in
291 testes size (Firman and Simmons 2010; Firman *et al.* 2011). Moreover, the increase in
292 sperm production resulted in enhanced success in sperm competition (Firman and
293 Simmons 2011). We do not have testes size data from males from the 24th generation,
294 however testes size had not diverged after eight (Firman and Simmons 2010), 16
295 (Firman *et al.* 2011) or 18 generations of selection. Thus, it is likely the increase in
296 sperm production among males from the polygamous lines is attributable to an
297 increase in the proportion of sperm-producing tissue, which was documented here.

298 Sperm production is also influenced by the efficiency of the sperm-producing
299 tissue, for example the rate at which individual sperm can be manufactured. In
300 mammals, the duration of spermatogenesis is defined as the ‘seminiferous epithelium
301 cycle length’ (SECL), describing one complete series of cell associations that occurs
302 in the seminiferous epithelium (Clermont 1972). Across species, variation in SECL
303 has been shown to correlate negatively with relative testes size, supporting the idea
304 that sperm competition selects for a faster rate of spermatogenesis (Pierce and Breed
305 2001; Paraponov et al. 2008; Ramm and Stockley 2010). In addition to evolutionary
306 responses, variation in the speed of spermatogenesis might also account for the
307 adaptive plasticity in sperm production that has been observed in response to local
308 sperm competition conditions (delBarco-Trillo and Ferkin 2004; Ramm and Stockley
309 2009; Firman et al. 2013). Our findings on house mice have important implications
310 for the general assumption that larger testes confer greater rates of sperm production,
311 and emphasise the need for explorations beyond a simple measure of testes size and
312 into testes tissue organisation and function (Ramm and Schärer 2014).

313 As almost all male sex hormone production occurs in the testes interstitium
314 (Stocco and McPhaul 2006), we contend that an increase in the proportion of
315 seminiferous tubules and a reduction in the amount of interstitial tissue could have
316 important implications for precopulatory sexual selection. Testosterone, which is
317 produced by the Leydig cells, plays a critical role in the promotion of secondary
318 sexual characters (Dufau 1996). For example, testosterone-dependent urine scent-
319 marking behaviour in male house mice is indicative of dominance status and territory
320 defense (Desjardins et al. 1973; Hurst 1990), and is assessed by females to gauge the
321 quality of potential mates (Wolff 1985, Penn and Potts 1998). Variation in
322 testosterone levels can lead to changes in the major urinary proteins that mediate the

323 release of pheromones (Harvey et al. 1989). Therefore, a reduction in testes interstitial
324 tissue, and consequently a reduction in the density of Leydig cells, could result in a
325 reduction in testosterone production and influence the quality or frequency of scent-
326 marks produced by males. A recent study has revealed that sexually receptive females
327 spent more time associating with males from the monogamous populations compared
328 to males from the polygamous populations, suggesting that these males have a
329 precopulatory advantage which could be attributable to an intrinsic quality associated
330 with the scents that they produce (Firman 2014). The divergence in testes tissue
331 composition among males evolving with and without sperm competition reported here
332 certainly warrants further research into evolutionary trade-offs between pre- and
333 postcopulatory sexually selected traits.

334 In summary, we have documented divergence in testes tissue composition
335 among house mice from populations that had evolved under a polygamous or
336 monogamous mating regime for 24 generations. Our statistical analysis revealed that
337 males that had evolved with sperm competition had testes with a significantly greater
338 proportion of seminiferous tubules compared with males that had evolved under
339 enforced monogamy. Our finding accounts for the previously reported divergence in
340 sperm number and sperm competitiveness that had been observed in the absence of
341 evolutionary changes in testes size (Firman and Simmons 2010; Firman and Simmons
342 2011). This study has important implications for the general, mostly untested
343 assumption that testes size is a strong predictor of sperm production, and emphasizes
344 the need for future research to explore the evolutionary implications of sperm
345 competition for testicular efficiency.

346

347

348 **ACKNOWLEDGEMENTS**

349 We thank Tom Stewart for assistance with histology, and Freddy Simmons and
350 Shresta Lobind for animal husbandry. This research was funded by the Australian
351 Research Council (RCF, FG-G, LWS). FG-G was also supported by the Spanish
352 Ministry of Economy through the Ramon y Cajal program and grant (cofunded by the
353 European Regional Development Fund) CGL2012-34685. The research was approved
354 by the UWA Animal Ethics Committee (07/100/607).

355

356 **LITERATURE CITED**

357 Almbro, M., and L. W. Simmons. 2014. Sexual selection can remove an
358 experimentally induced mutation load. *Evolution* 68:295–300.

359

360 Arbuthnott, D., and H. D. Rundle. 2012. Sexual selection is ineffectual or inhibits the
361 purging of deleterious mutations in *Drosophila melanogaster*. *Evolution* 66:2127–
362 2137.

363

364 Beatty, R. A. 1957. A pilot experiment with heterospermic insemination in the rabbit.
365 *J. Genet.* 55:325.

366

367 Becker, W. A. 1984. *Manual of quantitative genetics*. Pullman, Washington.

368

369 Birkhead, T. R., Hosken, D. J., and Pitnick, S. 2009. *Sperm biology. An evolutionary*
370 *perspective*. Academic Press, Burlington MA.

371

- 372 Boschetto, C., C. Gasparini, and A. Pilastro. 2011. Sperm number and velocity affect
373 sperm competition success in the guppy (*Poecilia reticulata*). Behav. Ecol. Sociobiol.
374 65:813–821.
375
- 376 Brown, C.R., and M. B. Brown. 2003. Testis size increases with colony size in cliff
377 swallows. Behav. Ecol. 14: 569–575.
378
- 379 Byrne, P. G., J. D. Roberts and L. W. Simmons. 2002. Sperm competition selects for
380 increased testes mass in Australian frogs. J. Evol. Biol. 15:347–355.
381
- 382 Lee, C. 1976. Agonistic behavior, sexual attraction, and olfaction in mice. Pp. 161–
383 180 in R. Doty, ed. Mammalian Olfaction, Reproductive Processes and Behaviour.
384 Academic Press, London.
385
- 386 Clermont, Y. 1972. Kinetics of spermatogenesis in mammals: seminiferous
387 epithelium cycle and spermatogonial renewal. Physiol. Rev. 52:198–236.
388
- 389 Dean, M. D., K. G. Ardlie, and M. W. Nachman. 2006. The frequency of multiple
390 paternity suggests that sperm competition is common in house mice (*Mus*
391 *domesticus*). Mol. Ecol. 15:4141–4151.
392
- 393 delBarco-Trillo, J., and M. H. Ferkin. 2004. Male mammals respond to a risk of
394 sperm competition conveyed by odours of conspecific males. Nature 431:446–449.
395

396 Desjardins C., J. A. Maruniak, F. H. Bronson. 1973. Social rank in house mice:
397 differentiation revealed by ultraviolet visualization of urinary marking patterns.
398 Science 182:939–941.
399
400 Dufau, M. 1996. The lutenizing hormone receptor. Pp. 333–350 in A. H. Payne, M. P.
401 Hardy, and L. D. Russell, eds. The Leydig Cell. Cache River Press, Illinois.
402
403 Dzminiski, M. A., J. D. Roberts, M. Beveridge, and L. W. Simmons. 2009. Among
404 population covariation between sperm competition and ejaculate expenditure in frogs.
405 Behav. Ecol. 21:322–328.
406
407 Fuentes, L. B., N. Caravaca, L. E. Pelzer, L. A. Scardapane, R. S. Piezzi, and J. A.
408 Guzman. 1991. Seasonal variations in the testes and epididymis of vizcacha
409 (*Lagostomus maximus maximus*). Biol. Reprod. 45:493–497.
410
411 Firman, R. C. 2014. Female social preference for males that have evolved via
412 monogamy: evidence of a trade-off between pre- and post-copulatory sexually
413 selected traits? Biol. Lett. 10:20140659.
414
415 Firman, R. C., and L. W. Simmons. 2008. The frequency of multiple paternity
416 predicts variation in testes size among island populations of house mice. J. Evol. Biol.
417 21:1524–1533.
418
419 Firman R.C., and L. W. Simmons. 2010. Experimental evolution of sperm quality via
420 postcopulatory sexual selection in house mice. Evolution 64:1245–1256.

421

422 Firman, R. C., and L. W. Simmons. 2011. Experimental evolution of sperm
423 competitiveness in a mammal. *BMC Evol. Biol.* 11:19.

424

425 Firman, R. C., L. Y. Cheam, and L. W. Simmons. 2011. Sperm competition does not
426 influence sperm hook morphology in selection lines of house mice. *J. Evol. Biol.*
427 24:856–862.

428

429 Firman, R. C., I. Klemme, and L. W. Simmons. 2013. Strategic adjustments in sperm
430 production within and between two island populations of house mice. *Evolution*
431 67:3061–3070.

432

433 Gage, M. J. G. 1994. Associations between body size, mating pattern, testis size and
434 sperm lengths across butterflies. *Proc. R. Soc. Lond. B.* 258:247–254.

435

436 Gage, M. J. G., and E. H. Morrow. 2003. Experimental evidence of the evolution of
437 numerous, tiny sperm via sperm competition. *Curr. Biol.* 13:754–757.

438

439 Gier, H. T., and G. B. Marion. 1970. Development of the mammalian testis. Pp. 1–45
440 in A. D. Johnson, W. R. Gomes, and N. L. Vandemark, eds. *The testis*. Academic
441 Press, New York.

442

443 Goldberg, S. R., and W. S. Parker. 1975. Seasonal testicular histology of the colubrid
444 snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. *Herp.* 31:317–322.

445

- 446 Gomendio, M., A. H. Harcourt, and E. R. S. Roldan. Sperm competition in mammals.
447 Pp. 667–755 in T.R. Birkhead, and A. P. Møller, eds. Sperm competition and sexual
448 selection. Academic Press, San Diego.
- 449
- 450 Harcourt, A. H., P. H. Harvey, S. G. Larson, and R. V. Short. 1981. Testis weight,
451 body weight and breeding system in the primates. *Nature* 293:55–57.
- 452
- 453 Harvey, S., J. Bozana, and M. Novotny. 1989. Pattern of volatile compounds in
454 dominant and subordinate male mouse urine. *J. Chem. Ecol.* 15:2061–2072.
- 455
- 456 Hollis, B., J. L. Fierst, and D. Houle. 2009. Sexual selection accelerates the
457 elimination of a deleterious mutant in *Drosophila melanogaster*. *Evolution* 63:324–
458 333.
- 459
- 460 Hosken, D. J. 1997. Sperm competition in bats. *Proc. R. Soc. Lond. B* 264:385–392.
- 461
- 462 Hosken, D. J., and P. I. Ward. 2001. Experimental evidence for testis size evolution
463 via sperm competition. *Ecol. Lett.* 4:10–13.
- 464
- 465 Hurst, J. L. 1990. Urine marking in populations of wild house mice *Mus domesticus*
466 Ruttly. I. Communication between males. *Anim. Behav.* 40:209–222.
- 467
- 468 Lüpold, S., G. M. Linz, J. W. Rivers, D. F. Westneat, and T. R. Birkhead. 2009.
469 Sperm competition selects beyond relative testes size in birds. *Evolution* 63:391–402.

470

471 Martin, P. A., T. J. Reimers, J. R. Lodge, and P. J. Dzuik. 1974. The effect of ratios
472 and numbers of spermatozoa mixed from two males on proportions of offspring. J.
473 Reprod. Fertil. 39:251–258.

474

475 Mayhew, W. W., and S. J. Wright. 1970. Seasonal changes in testicular histology of
476 three species of the lizard genus *Uma*. J. Morph. 130:163–185.

477

478 McLearn, K. R., and J. T. Manning. 1985. Mate choice, monogamy and mutational
479 load. J. Theor. Biol. 116:369–376.

480

481 Montoto, L. G., M. Concepción, M. Tourmente, J. Martin-Coello, C. Crespo, J. J.
482 Luque-Larena, M. Gomendio, and E. R. S. Roldan. 2011. Sperm competition, sperm
483 numbers and sperm quality in muroid rodents. PLoS One 6:e18173.

484

485 Montoto, L. G., L. Arregui, N. M. Sanchez, M. Gomendio, and E. R. S. Roldan. 2012.
486 Postnatal testicular development in mouse species with different levels of sperm
487 competition. Reproduction 143: 333–346.

488

489 Parapanov, R., S. Nussle, J. Hausser, and P. Vogel. 2008. Relationships of basal
490 metabolic rate, relative testis size and cycle length of spermatogenesis in shrews
491 (Mammalia, Soricidae). Reprod. Fertil. Dev. 20:431–439.

492

493 Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the
494 insects. Biol. Rev. 45:525–567.
495

496 Parker G. A., and T. Pizzari. 2010. Sperm competition and ejaculate economics. Biol.
497 Rev. 85:897–934.
498

499 Penn, D. J., and W. K. Potts. 1998. Chemical signals and parasite-mediated sexual
500 selection. TREE 13: 391–396.
501

502 Pierce, E. J., and W. G. Breed. 2001. A comparative study of sperm production in two
503 species of Australian arid zone rodents (*Pseudomys australis*, *Notomys alexis*) with
504 marked differences in testis size. Reproduction 121:239–247.
505

506 Pitcher, T. E., P. O. Dunn, and L. A. Whittingham. 2005. Sperm competition and the
507 evolution of testis size in birds. J. Evol. Biol. 18:557–567.
508

509 Pitnick, S., G. T. Miller, J. Reagan, and B. Holland. 2001. Males’ evolutionary
510 response to experimental removal of sexual selection. Proc. R. Soc. Lond. B
511 268:1071–1080.
512

513 Radwan, J. 2004. Effectiveness of sexual selection in removing mutations induced
514 with ionization. Ecol. Let. 7:1149–1154.
515

516 Ramm, S. A., and L. Schärer. 2014. The evolutionary ecology of testicular function:
517 size isn’t everything. Biol. Rev. doi:10.1111/brv.12084

518

519 Ramm, S. A., and P. Stockley. 2009. Adaptive plasticity of mammalian sperm
520 production in response to social experience. *Proc. R. Soc. Lond. B* 276:745–751.

521

522 Ramm, S. A., and P. Stockley. 2010. Sperm competition and sperm length influence
523 the rate of mammalian spermatogenesis. *Biol. Lett.* 6:219–221.

524

525 Hochereau-de Reviers, M. H., and G. A. Lincoln. 1978. Seasonal variation in the
526 histology of the testis of the red deer, *Cervus elaphus*. *J. Reprod. Fert.* 54:209–213.

527

528 Ribble, D. O., and J. S. Millar. 1992. Intraspecific variation in testes size among
529 northern populations of *Peromyscus*. *Funct. Ecol.* 6:455–459.

530

531 Roosen-Runge, E. C. 1977. The process of spermatogenesis in mammals. Cambridge
532 University Press, Cambridge.

533

534 Rowe, M., and S. Pruett-Jones. 2011. Sperm competition selects for sperm quantity
535 and quality in the Australian Maluridae. *PLoS One* 6:e15720.

536

537 Russell, L. D., H. P. Ren, I. S. Hikim, W. Schulze, and A. P. S. Hikim. 1990. A
538 comparative study in twelve mammalian species of volume densities, volumes, and
539 numerical densities of selected testis components, emphasizing those related to the
540 Sertoli cell. *Am. J. Anat.* 188:21–30.

541

542 Sawyer, T. F. 1980. Androgen effects on responsiveness to aggression and stress-

543 related odors of male mice. *Physiol. Behav.* 25:183–187.

544

545 Schärer L., P. Ladurner, and R. M. Rieger. 2004. Bigger testes do more work:

546 experimental evidence that testis size reflects testicular cell proliferation activity in

547 the marine invertebrate, the free-living flatworm *Macrostomum* sp. *Behav. Ecol.*

548 *Sociobiol.* 56:420–425.

549

550 SciStat Calc. 2013. Levene's Test: equality of variances. Available at:

551 scistatcalc.blogspot.com. Accessed 2nd November, 2014.

552

553 Shabalina, S. A., L. Y. Yampolsky, and A. S. Kondrashov. 1997. Rapid decline of

554 fitness in panmictic populations of *Drosophila melanogaster* maintained under

555 relaxed natural selection. *Proc. Natl. Acad. Sci. USA* 94:13034–13039.

556

557 Simmons, L. W. 2001. *Sperm competition and its evolutionary consequences in*

558 *insects*. Princeton University Press, Princeton.

559

560 Simmons, L. W., and F. Garcia-Gonzalez. 2008. Evolutionary reduction in testes size

561 and competitive fertilisation success in response to the experimental removal of

562 sexual selection in dung beetles. *Evolution* 62:2580–2591.

563

564 Stocco, D. M., and M. J. McPhaul. 2006. Physiology of testicular steroidogenesis. Pp.

565 977–1016 in J. D. Neill, T. M. Plant, D. W. Pfaff, J. R. G. Challis, D. M. De Krester,

566 Richards, J.S., and P. M. Wassarman, eds. *Knobil and Neill's Physiology of*

567 *Reproduction*. Vol. 1, Third Edition. Academic Press, Burlington.

568

569 Vrech D. E., P. A. Olivero, C. I. Mattoni, and A. V. Peretti. 2014. Testes mass, but
570 not sperm length, increases with higher levels of polyandry in an ancient sex model.
571 PLoS ONE 9:e94135.

572

573 Whitlock, M. C., and A. F. Agrawal. 2009. Purging the genome with sexual selection:
574 reducing mutation load through selection on males. *Evolution* 63:569–582.

575

576 Wistuba, J., A. Schrod, B. Grieve, J. K. Hodges, H. Aslam, G. F. Weinbauer, and C.
577 M. Luetjens. 2003. Organization of seminiferous epithelium in primates: relationship
578 to spermatogenic efficiency, phylogeny, and mating system. *Biol. Reprod.* 69:582–
579 591.

580

581 Wistuba, J. A., J. Stukenborg, and M. Luetjens. 2007. Mammalian spermatogenesis.
582 *Funct. Develop. Embrol.* 1:99–117.

583

584 Wolff, R. J. 1985. Mating behaviour and female choice: their relation to social status
585 in wild caught house mice *Mus musculus* housed in a semi-natural environment. *J.*
586 *Zool.* 207:43–51.

587

588

589

590

591

592

593 **Table 1.** ANOVA comparing the proportion of sperm-producing tissue in the
594 testes of males that have evolved under either a polygamous or monogamous
595 selection regime.

596

Effect	SS	df	MS	<i>F</i>	<i>P</i>
Selection history	0.034	1	0.033	32.602	0.005
Line[selection history]	0.004	4	0.001	0.243	0.913
Error	0.223	54			

597

598

599

600

601 **Figure 1.** The mean proportion of seminiferous tubules in the testes of house
602 mice from monogamous (3) and polygamous (3) selection lines (as measured
603 from images of histological preparations).

604

605

606

607 **Figure 2.** Example images displaying the difference in the density of
608 seminiferous tubules in the testes of house mice from monogamous (1-3) and
609 polygamous (4-6) selection lines.

Figure 1.

Figure 2.