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3 **EXPERIMENTAL EVIDENCE FOR THE**
4 **EVOLUTION OF THE MAMMALIAN BACULUM**
5 **BY SEXUAL SELECTION**

6

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13 Running title: Evolution of the mammalian baculum

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15 Data will be archived at DRYAD

16 Male genitalia exhibit a taxonomically widespread pattern of rapid and
17 divergent evolution. Sexual selection is generally believed to be responsible
18 for these patterns of evolutionary divergence, although empirical support for
19 the sexual selection hypothesis comes mainly from studies of insects. Here
20 we show that sexual selection is responsible for an evolutionary divergence in
21 baculum morphology among populations of house mice *Mus domesticus*. We
22 sourced mice from three isolated populations known to be subject to differing
23 strengths of postcopulatory sexual selection and bred them under common-
24 garden conditions. Mice from populations with strong postcopulatory sexual
25 selection had bacula that were relatively thicker compared with mice from
26 populations with weak selection. We used experimental evolution to
27 determine whether these patterns of divergence could be ascribed to
28 postcopulatory sexual selection. After 27 generations of experimental
29 evolution, populations of mice subjected to postcopulatory sexual selection
30 evolved bacula that were relatively thicker than populations subjected to
31 enforced monogamy. Our data thereby provide evidence that postcopulatory
32 sexual selection underlies an evolutionary divergence in the mammalian
33 baculum, and supports the hypothesis that sexual selection plays a general
34 role in the evolution of male genital morphology across evolutionary diverse
35 taxonomic groups.

36

37 **KEY WORDS:** os penis, sperm competition, cryptic female choice,
38 experimental evolution, house mice, *Mus*

39 Male genitalia typically exhibit patterns of rapid and divergent evolution (Eberhard
40 1985). Sexual selection acting during and after copulation is thought to play a role in
41 genital evolution generally (Hosken and Stockley 2004), although empirical support
42 for the sexual selection hypothesis comes largely from studies of insects (Simmons
43 2014). In damselflies (Waage 1979), water striders (Arnqvist and Danielsson 1999;
44 Preziosi and Fairbairn 2000), beetles (House and Simmons 2003; Rodriguez et al.
45 2004; Simmons et al. 2009; Hotzy et al. 2012), mantids (Holwell et al. 2010) and
46 earwigs (van Lieshout and Elgar 2011), male genital morphology influences mating,
47 insemination, or fertilization success, and comparative analyses show that species of
48 insects subject to greater sexual selection have more exaggerated and complex genital
49 morphology (Arnqvist 1998). Direct evidence that sexual selection can drive the
50 evolution of insect genital morphology comes from just two studies of experimental
51 evolution in which manipulation of the intensity of sexual selection was shown to
52 generate divergence in male and female genital morphology among populations of
53 dung beetles (Simmons et al. 2009; Simmons and Garcia-Gonzalez 2011) and seed
54 beetles (Cayetano et al. 2011). Direct evidence that sexual selection acts on vertebrate
55 genitalia is considerably more limited (Langerhans et al. 2005; Evans et al. 2011;
56 Mautz et al. 2013).

57 Typical of male genital morphology generally, the mammalian baculum or
58 penis bone exhibits considerable interspecific variation in size, shape and complexity
59 (Patterson and Thaler 1982; Dixson 2012). Despite years of speculation, the selective
60 pressures underlying divergence in baculum morphology remains one of the most
61 puzzling enigmas of mammalian morphology (Larivière and Ferguson 2002; Ramm
62 2007; Stockley 2012). At least three hypotheses have been proposed to explain
63 baculum evolution. The structure may impart rigidity to facilitate intromission (Long

64 1969), assist in the transport of sperm during prolonged copulations (Dixson 2012), or
65 stimulate the reproductive tract and induce ovulation (Greenwald 1956; Eberhard
66 1985). These hypotheses share the common prediction that baculum morphology
67 should be subject to sexual selection through variation in mating success and/or
68 through sperm competition, cryptic female choice and sexual conflict (Eberhard 1993;
69 Hosken and Stockley 2004). However, evidence for sexual selection acting on the
70 mammalian baculum has been largely indirect and equivocal.

71 Under the assumption that traits subject to sexual selection should exhibit
72 positive allometry, the scaling relationships between baculum length and body size
73 have been explored, but have produced conflicting results (Patterson 1983; Lüpold et
74 al. 2004; Miller and Nagorsen 2008; Ramm et al. 2010; Schulte-Hostedde et al. 2011).
75 The conceptual premise on which these studies have been based has also been
76 questioned (Bonduriansky 2007). There is some evidence from comparative studies of
77 rodents and carnivores (Ramm 2007) that baculum length increases with testes mass,
78 a commonly used proxy for the strength of postcopulatory sexual selection (Simmons
79 and Fitzpatrick 2012). A recent comparative study of pinnipeds provided support for
80 the view that baculum length is subject to rapid evolutionary change among species
81 with stronger sexual selection, and also found a positive evolutionary association
82 between testes size and baculum length (Fitzpatrick et al. 2012). However, studies of
83 bats (Hosken et al. 2001) and primates (Ramm 2007) have found no associations
84 between testes size and baculum length. Therefore, the role of sexual selection in
85 driving these patterns of covariation remains equivocal, especially since testes size is
86 not necessarily a reliable proxy for aspects of postcopulatory sexual selection via
87 cryptic female choice or sexual conflict (Ramm 2007). Unequivocal support for the
88 role of sexual selection in driving bacula divergence requires the demonstration that

89 baculum morphology varies directly in response to the strength of sexual selection.
90 The best evidence thus far that baculum morphology may be subject to sexual
91 selection comes from a recent study in which the width of the baculum was found to
92 predict male reproductive success in small experimental populations of house mice,
93 *Mus musculus domesticus* (Stockley et al. 2013). Here, we conducted comparisons
94 among natural populations of house mice, and used experimental evolution to provide
95 direct evidence that sexual selection can drive the evolutionary divergence of baculum
96 morphology.

97 Isolated populations of house mice occur on offshore islands along the coast of
98 Western Australia. Previously, we used microsatellite genotyping to estimate the rates
99 of mixed paternity within litters produced by females sampled from seven populations
100 (Firman and Simmons 2008). We found significant covariation between rates of
101 mixed paternity and testes size across these populations, consistent with the
102 theoretical expectation that among populations and species increased strength of
103 postcopulatory sexual selection should favor increased male expenditure on sperm
104 production (Parker and Pizzari 2010). If postcopulatory sexual selection is responsible
105 for the evolution of baculum morphology we might expect to see differences in
106 baculum morphology among these populations. Nonetheless, correlational data such
107 as these remain ambiguous because factors instead of, or as well as, the strength of
108 postcopulatory sexual selection might impact male genital development. We therefore
109 adopted the powerful approach of experimental evolution to determine whether sexual
110 selection can indeed generate the patterns of divergence in male genital morphology
111 we observed among natural mouse populations.

112 We imposed postcopulatory sexual selection on four replicate populations of
113 wild-derived house mice by ensuring females mated with three males in quick

114 succession within a single estrous cycle, and prevented postcopulatory sexual
115 selection in four replicate populations by enforcing monogamy (Firman and Simmons
116 2010b). These populations had diverged in important postcopulatory fitness traits
117 such as sperm quality (Firman and Simmons 2010b) and competitive fertilization
118 success (Firman and Simmons 2010a). If postcopulatory sexual selection underlies the
119 evolutionary divergence in genital morphology we would expect these experimental
120 populations to show divergence in baculum morphology.

121

122 *Methods*

123 **AMONG POPULATION VARIATION**

124 We sampled male (20) and female (20) mice from three isolated populations that
125 occur on islands located off the coast of Western Australia; Rat Island (28°42'S,
126 113°47'E), Boullanger Island (30°19'S, 115°00'E) and Whitlock Island (30°19'S,
127 114°59'E). These populations vary in the strength of postcopulatory sexual selection,
128 having high (71%), medium (31%) and low (17%) levels of mixed paternity
129 respectively (Firman and Simmons 2008). Mice were trapped in Elliot small mammal
130 traps baited with peanut butter and rolled oats. The mice were returned to the
131 University of Western Australia and outbred under common-garden conditions for
132 three generations before baculum morphology was assessed using geometric
133 morphometric analysis (Zelditch et al. 2004). Common-garden rearing was adopted in
134 order to eliminate any environmental factors that might influence phenotype, and thus
135 uncover variation in baculum morphology attributable to genetic divergence.

136 Mice were maintained in the same constant temperature room at 25°C with a
137 14:10 h light:dark regime. The animals were housed in polyurethane boxes (12 cm ×
138 12 cm × 30 cm), lined with chaff, and with wire lids. Rodent feed and water were

139 provided *ad libitum*. For breeding, a male and female were housed in the same box.
140 After two weeks, the pairs were checked twice a week and the males were removed
141 when the female was seen to be pregnant. Litters were weaned at three weeks of age
142 and housed in a single box as sibling groups for two weeks, after which males were
143 housed individually and females were housed in sister pairs. Matings were conducted
144 when mice were two months of age, and males held for a further two weeks after
145 mating before being euthanased and frozen at -20°C. Thus, all males were aged ten
146 weeks and had mated with a single female when they were assessed for baculum
147 morphology.

148

149 **EXPERIMENTAL EVOLUTION**

150 Full details of the source populations, and the experimental procedures adopted during
151 experimental evolution have been published elsewhere (Firman and Simmons 2010b).
152 In brief, a wild-derived colony of house mice was established at the Animal
153 Resources Centre (ARC) (Murdoch, WA) with 90 breeding pairs sourced from six
154 wild, Australian *Mus domesticus* populations. The colony was maintained as an
155 outbred population under the Poiley outbreeding system, after which the outbreeding
156 regime changed to a dedicated program based on the coefficient of inbreeding. After
157 approximately 30 generations of enforced monogamy, sexually mature mice (n = 120)
158 were transported to, and held at the University of Western Australia. Male/female
159 pairs (n = 60) reproduced to establish a base population from which our selection
160 lines were derived. We referred to the ARC colony pedigree to ensure that close
161 relatives did not breed (Firman and Simmons 2010b).

162 We established eight populations of mice and randomly assigned them to
163 treatments that would evolve with or without postcopulatory sexual selection. We

164 imposed postcopulatory sexual selection on four replicate populations by ensuring
165 females mated with three males in quick succession within a single estrous cycle, and
166 prevented postcopulatory sexual selection in four replicate populations by providing
167 each female with only a single male (Firman and Simmons 2010b). In all other
168 respects these populations were identical, and maintained following the common-
169 garden protocol outlined above. Precopulatory sexual selection was removed via the
170 random assignment of males to females at the time of mating, and natural selection
171 was removed by allowing each of 18 families within each population to contribute the
172 same number of offspring to subsequent generations. The effective population sizes
173 were 18 females and 18 males for monogamous populations and 18 females and
174 potentially <18 males for polygamous populations because not all males were
175 expected to contribute equally due to postcopulatory sexual selection. Polygamous
176 populations might thereby be subject to greater risk of inbreeding. However, the
177 increased litter size (Firman and Simmons 2010b), sperm quality and quantity
178 (Firman and Simmons 2010b), male competitive fertility (Firman and Simmons
179 2010a), and increased viability of embryos sired by males from polygamous lines
180 (Firman and Simmons 2012), indicates that these populations did not suffer from
181 inbreeding depression.

182 To assess divergence in baculum morphology, we sampled 79 sexually mature
183 males (10 weeks of age) after two generations of selection and 71 sexually mature
184 males after 27 generations of selection. Each male was taken from a different family,
185 and from families distributed across the eight experimentally evolving populations
186 (mean±SE number of males per population and generation 9±0.5).

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190 **GEOMETRIC MORPHOMETRICS**

191 We weighed each male to obtain an overall estimate of body size. We did not use a
192 linear measure of body size, such as foot length, because different morphological
193 traits scale differently with body size, confounding any estimate of baculum allometry
194 with the arbitrarily chosen trait's own scaling relationship with body size. We
195 converted body mass to a linear scale by taking the cube root.

196 Mice were thawed and bacula prepared following established protocols
197 (Ramm et al. 2010). Digital images were captured using a binocular microscope at
198 $\times 25$ magnification. Geometric morphometrics were conducted using the software
199 developed by F. James Rohlf (Rohlf 2009). A total of 36 landmarks (2 fixed) were
200 placed around the periphery of the baculum using the software tpsDig2 v2.12 (Fig. S1
201 in the online supporting information). The software Tpsrelw v1.46 was then used to
202 extract relative warps and centroid sizes. In brief, the analysis determines the average
203 positions of each landmark and calculates a consensus shape based on all samples.
204 Relative warps represent axes of variation in the partial warps (each landmark's
205 position relative to that landmark's consensus position) (Zelditch et al. 2004). We only
206 considered RWs that individually explained $>10\%$ of the variance in baculum shape.
207 A measure of size is provided by centroid size (the square root of the summed
208 distances of each landmark from the average or centroid position, Zelditch et al.
209 2004). We assessed the accuracy in extracting measures of baculum size and shape by
210 conducting geometric morphometric procedures on the bacula of 10 males on two
211 separate occasions. Thus we placed the same baculum under the microscope twice,
212 and placed landmarks on the independent images obtained. Measures of baculum size

213 and shape were significantly repeatable (Centroid size: $F_{9, 10}=3.78$, $P=0.025$, $R=0.735$;
214 RW1: $F_{9, 10}=37.30$, $P<0.001$, $R=0.973$; RW2: $F_{9, 10}=23.22$, $P<0.001$, $R=0.957$).

215

216 *Results*

217 **POPULATION DIVERGENCE**

218 Descriptive statistics for body size and genital traits can be found in Table S1 of the
219 online supporting material. We found significant divergence in baculum size among
220 the three island populations of house mice that we sampled (Fig. 1). We conducted
221 ANCOVA using \log_{10} baculum centroid size as the dependent variable and \log_{10} cube
222 root of body mass, population, and their interaction as main effects. Baculum size
223 increased with body size but there was no significant interaction between body size
224 and population ($F_{2, 119}=0.45$, $P=0.639$) indicating that baculum allometry did not vary
225 among populations. The interaction effect was thus removed from the statistical
226 model, yielding a common allometric slope of 0.355 ± 0.089 ($F_{1, 121}=16.09$, $P<0.001$).
227 There was also significant variation in baculum size among populations ($F_{2, 121}=20.61$,
228 $P<0.001$). Posthoc tests (Tukey HSD) revealed that males from the Rat Island
229 population, within which postcopulatory sexual selection is strongest, had a smaller
230 baculum than males from the other populations (Fig. 1).

231 We also found evidence of significant divergence among populations in
232 baculum shape (Fig. 2). The first three Relative Warps (RW) from our geometric
233 morphometric analysis explained 46.4%, 15.2% and 11.6% of the variance in
234 baculum shape respectively. The first and second RWs described variation in the
235 relative thickness of the basal bulb of the baculum, and in the relative length and
236 thickness of its shaft (Fig. 2). RW3 described subtle differences in the shape of the
237 basal bulb (Fig. S2). We did not consider RW3 further as none of the variation in this

238 RW could be explained by our predictor variables. There were no significant
239 interactions between population and male size for either RW (RW1: $F_{2, 119}=0.39$,
240 $P=0.679$; RW2: $F_{2, 119}=0.26$, $P=0.768$) so interaction effects were removed from the
241 statistical models. Male size had no influence on baculum shape (RW1: $F_{1, 121}=2.08$,
242 $P=0.152$; RW2: $F_{1, 121}=2.36$, $P=0.127$) which varied significantly among populations
243 (RW1: $F_{2, 121}=60.05$, $P<0.001$; RW2: $F_{2, 121}=3.53$, $P=0.033$). Posthoc tests indicated
244 that baculum thickness was relatively greater in males from populations with stronger
245 postcopulatory sexual selection (Fig. 2).

246

247 **DIVERGENCE UNDER EXPERIMENTAL EVOLUTION**

248 Descriptive statistics for body size and genital traits of mice from our experimental
249 populations can be found in Tables S2 and S3 of the online supporting information.
250 Geometric morphometric analysis returned qualitatively similar shape variables to
251 those found among natural populations of mice. The first two RWs explained 33.5%
252 and 28.3% of the variation in baculum shape and described variation in the relative
253 thickness of the basal bulb of the baculum, and in the relative length and thickness of
254 its shaft (Fig. 3). Thus, the major axes of variation obtained from the two geometric
255 morphometric analyses of the bacula of mice from natural populations and from
256 experimental populations described qualitatively similar patterns of baculum shape
257 variation (contrast thin-plate splines in Fig. 2 and 3).

258 We conducted nested ANOVAs on the size and shape variables, entering
259 selection history (with or without postcopulatory sexual selection) as the main effect,
260 and replicate population nested within selection history as a random effect. We also
261 entered \log_{10} cube root body mass as a covariate (see Tables S4 and S5 in the online
262 supporting information). After two generations of experimental evolution the

263 allometric slope of baculum size on body size was 0.272 ± 0.106 ($F_{1,70}=6.63$, $P=0.012$)
264 and males from the polygamous populations had relatively larger bacula than did
265 males from the monogamous populations ($F_{1,6}=22.32$, $P<0.001$; least square mean log
266 centroid size after adjusting for body size, monogamous 3.36 ± 0.002 vs polygamous
267 3.37 ± 0.002). The populations did not differ in baculum shape (RW1: $F_{1,6}=3.20$,
268 $P=0.111$; RW2: $F_{1,6}=0.81$, $P=0.397$) (Fig. 3 and Table S4 in the online supporting
269 information).

270 Following 27 generations of experimental evolution there was no longer
271 variation in baculum size among our experimental populations. The allometric slope
272 of baculum size on body size was 0.353 ± 0.090 ($F_{1,62}=15.29$, $P<0.001$) and baculum
273 size did not differ among monogamous and polygamous populations ($F_{1,6}=3.33$,
274 $P=0.113$, Table S5). However, the major axis of variation in baculum shape had
275 diverged in response to our manipulation of postcopulatory sexual selection; RW1
276 increased with male size ($F_{1,62}=4.14$, $P=0.046$) and differed significantly between
277 populations evolving under polygamy or enforced monogamy ($F_{1,6}=10.46$, $P=0.015$).
278 Males from polygamous populations that were subject to postcopulatory sexual
279 selection evolved relatively thicker bacula (Fig. 3 and Table S5 in the online
280 supporting information). Neither male size ($F_{1,62}=0.29$, $P=0.592$) nor selection history
281 ($F_{1,6}=0.39$, $P=0.551$) explained any of the variation in RW2 (Table S3 and S5).

282

283 **DISCUSSION**

284 We have documented significant variation in the size and shape of the baculum
285 among three isolated populations of house mice. Mice from populations with higher
286 rates of mixed paternity, and thus stronger postcopulatory sexual selection, had both
287 smaller and relatively thicker bacula. These patterns of variation were evident after

288 common-garden rearing indicating that the populations have diverged genetically in
289 baculum morphology. Among population variation in male genital morphology has
290 been reported from a number of rodent species (Simson et al. 1993; Good et al. 2003),
291 and also for guppies (*Poecilia reticulata*) and millipedes (*Antichiropus variabilis*)
292 (Evans et al. 2011; Wojcieszek and Simmons 2012). In guppies this variation is
293 associated with variation among populations in the prevalence of forced copulations
294 (Evans et al. 2011). While suggestive of a role for postcopulatory sexual selection,
295 correlational data such as these remain equivocal because these populations could be
296 subject to selection pressures other than postcopulatory sexual selection that might
297 impact male genital development. Our experimental populations of wild-derived
298 house mice, which were maintained under identical conditions with the exception that
299 they had evolved either in the presence or absence of postcopulatory sexual selection,
300 showed significant evolutionary divergence in baculum shape, but not size, after 27
301 generations of selection. These results show that the divergence in baculum size
302 observed among the natural populations of mice must have been due to selection
303 pressures in these populations that were absent in our experimental populations, such
304 as natural selection or precopulatory sexual selection. In contrast, our data confirm
305 that postcopulatory sexual selection underlies the divergence in the first major axis
306 describing baculum shape. We thereby provide direct experimental evidence for the
307 role of sexual selection in the evolution of the mammalian baculum.

308 Two previous experimental evolution studies have documented divergence in
309 male genital morphology following the experimental manipulation of sexual selection,
310 and both of these have been on insects. Using a similar approach, Simmons et al.
311 (2009) documented an evolutionary divergence in aedeagus shape, but not size,
312 among populations of dung beetles (*Onthophagus taurus*) evolving under either

313 polygamy or enforced monogamy, while in seed beetles (*Callosobruchus maculatus*)
314 Cayetano et al. (2011) found that males from monogamous populations evolved
315 shorter genital spines. In our study, experimental manipulation of the strength of
316 postcopulatory sexual selection acting on laboratory populations of house mice
317 generated an evolutionary divergence in male genital morphology that was
318 qualitatively similar to the patterns of divergence found among natural populations of
319 mice that differ in the strength of postcopulatory sexual selection. We thereby provide
320 evidence that sexual selection can contribute to the widely observed divergence in
321 mammalian genital morphology, and that sexual selection plays a general role in the
322 evolution of male genital morphology across diverse taxonomic groups (Eberhard
323 1985; Hosken and Stockley 2004).

324 Although we now have experimental evidence that divergence in baculum
325 morphology can be ascribed to sexual selection, future research should explore the
326 precise mechanism(s) by which baculum shape promotes male fitness. Several studies
327 of insect genitalia have revealed how aedeagus size and shape can impact male fitness
328 via their effects on mating and fertilization success (Arnqvist and Danielsson 1999;
329 House and Simmons 2003; Simmons et al. 2009; Holwell et al. 2010; Simmons and
330 Garcia-Gonzalez 2011; van Lieshout and Elgar 2011; Hotzy et al. 2012). Far less is
331 known of the fitness consequences of variation in baculum morphology for mammals.
332 Consistent with our findings, Stockley et al. (2013) have recently shown that the
333 thickness of the baculum base and shaft can predict male reproductive success in free-
334 ranging experimental populations of house mice. Collectively the evidence shows that
335 for house mice, baculum morphology does contribute to male fitness and can evolve
336 in response to postcopulatory sexual selection. Nonetheless, the precise mechanism by
337 which the baculum affects male fitness remains unknown.

338 Although our study is unable to identify the proximate mechanism by which
339 postcopulatory sexual selection acts, we can comment on the hypothesis that the
340 baculum is under sexual selection via its role in vaginal stimulation during copula
341 (Stockley et al. 2013). In house mice, the baculum forms the central core of the glans
342 penis and imparts rigidity to this structure which is itself covered with penile spines
343 capable of imparting vaginal stimulation (Rodriguez et al. 2011). Copulatory
344 stimulation is a marked feature of mating associations among rodents generally, and
345 variation among species in copulatory behaviour is associated with variation in
346 selection from sperm competition (Stockley and Preston 2004). In house mice, males
347 perform repeated intromissions without ejaculation, and this behavior is sensitive to
348 sperm competition risk (Preston and Stockley 2006). Differences in postcopulatory
349 sexual selection among our experimentally evolving populations has led to an
350 evolutionary divergence in mating behaviour; males from populations with
351 postcopulatory sexual selection have evolved greater mating durations (Klemme and
352 Firman 2013). Together, these findings strongly suggest that mechanical stimulation
353 of the female reproductive tract by males is the product of postcopulatory sexual
354 selection. In rats, the baculum increases stiffness of the penis during copulation by
355 transferring forces transmitted from the distal end of the penis to the corpus
356 cavernosum surrounding the basal bulb of the baculum (Kelly 2000). In this way, the
357 baculum can contribute directly to the stimulatory capacity of the penis.

358 In our experimentally evolving populations, postcopulatory sexual selection
359 also drove a divergence in litter size (Firman and Simmons 2010b). House mice
360 ovulate spontaneously (Hayssen et al. 1993) so it seems unlikely that vaginal
361 stimulation would influence ovulation in this species. Indeed, we found that increased
362 litter size among females that had evolved under postcopulatory sexual selection was

363 not due to changes in the number of ova released (Firman and Simmons 2010b).
364 Rather, males that had evolved under postcopulatory sexual selection were found to
365 stimulate higher rates of embryo implantation, and sire embryos with increased
366 viability (Firman and Simmons 2012). Therefore, we suggest that greater mechanical
367 stimulation of the female tract, achieved in part by a thickened baculum, might
368 promote greater neuroendocrine responses in the female that prepare for successful
369 embryo implantation and subsequent offspring development (Eberhard 1996; Yang et
370 al. 2009). Alternatively, vaginal stimulation may impact male fertilization success. In
371 house mice sexual stimulation during coitus has been shown to induce oviductal fluid
372 secretion which streams toward the uterus, and via rheotaxis guides the migration of
373 sperm to the site of fertilization (Miki and Clapham 2013). Males with thicker bacula
374 may stimulate greater or more rapid oviductal secretion, enhancing sperm rheotaxis
375 and subsequent fertilization success. Such differential responses by females to male
376 stimulation are key mechanisms of postcopulatory sexual selection via cryptic female
377 choice (Eberhard 1996). Our finding that postcopulatory sexual selection is a
378 significant factor driving the evolution of baculum shape should encourage greater
379 research effort into understanding the precise mechanisms underlying sexual selection
380 acting on the mammalian baculum.

381

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387 (07/100/607).

Supporting Information

The following supporting information is available for this article:

Figure S1. The baculum of a house mouse, *Mus domesticus*, showing the placement of 36 landmarks around its periphery for geometric morphometric analysis.

Landmarks 1 and 19, depicted in white, were assigned as fixed landmarks, while the remaining landmarks were assigned as sliding landmarks.

Figure S2. Variation in baculum shape described by RW3 among island populations of mice.

Table S1. Mean \pm SE body weight and genital measures (centroid size and relative warps) from three populations of house mice after 3 generations of common-garden rearing.

Table S2. Mean \pm SE body weight and genital size for populations evolving with (P) and without (M) postcopulatory sexual selection for 2 and 27 generations.

Table S3. Mean \pm SE relative warps ($\times 10^3$) describing baculum shape for populations evolving with (P) and without (M) postcopulatory sexual selection for 2 and 27 generations.

Table S4. Nested ANOVAs in the centroid size and shape (relative warps 1 & 2) of bacula among populations evolving with and without postcopulatory sexual selection for 2 generations.

Table S5. Nested ANOVAs in the centroid size and shape (relative warps 1 & 2) of bacula among populations evolving with and without postcopulatory sexual selection for 27 generations.

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565 **Figure 1.** Divergence in baculum size across three isolated populations of
566 house mice. The data presented are the allometric slopes of centroid size
567 extracted from geometric morphometric analyses plotted on body weight.

568

569 **Figure 2.** Divergence in baculum shape across three isolated populations of
570 house mice. Mean (\pm 95% CIs) relative warp scores (RW1: dark bars; RW2:
571 pale bars) both described variation in the thickness of the basal bulb of the
572 baculum and of the baculum shaft. Baculum shapes are visualised on the
573 right, and show the positions of the 36 landmarks around the periphery of the
574 baculum for extreme positive and negative values of each RW. The
575 consensus shape is displayed in the centre, with the two fixed landmarks
576 identified by open circles, and the sliding landmarks identified by solid circles.
577 Tukey HSD ($\alpha = 0.05$) revealed significant differences in RW1 between Rat
578 and Boullanger, and between Boullanger and Whitlock. For RW2 Boullanger
579 and Whitlock differed significantly, but neither population differed significantly
580 from Rat.

581

582 **Figure 3.** Divergence in baculum shape at generations 2 and 27 across four
583 replicate populations of house mice evolving with postcopulatory sexual
584 selection (P) and four replicate populations evolving under enforced
585 monogamy (M). Mean (\pm 95% CIs) scores on the first relative warp are
586 displayed, with the baculum shape visualised on the right by the positions of

587 landmarks associated with extreme positive (top) or negative (bottom) scores
588 on the relative warp.
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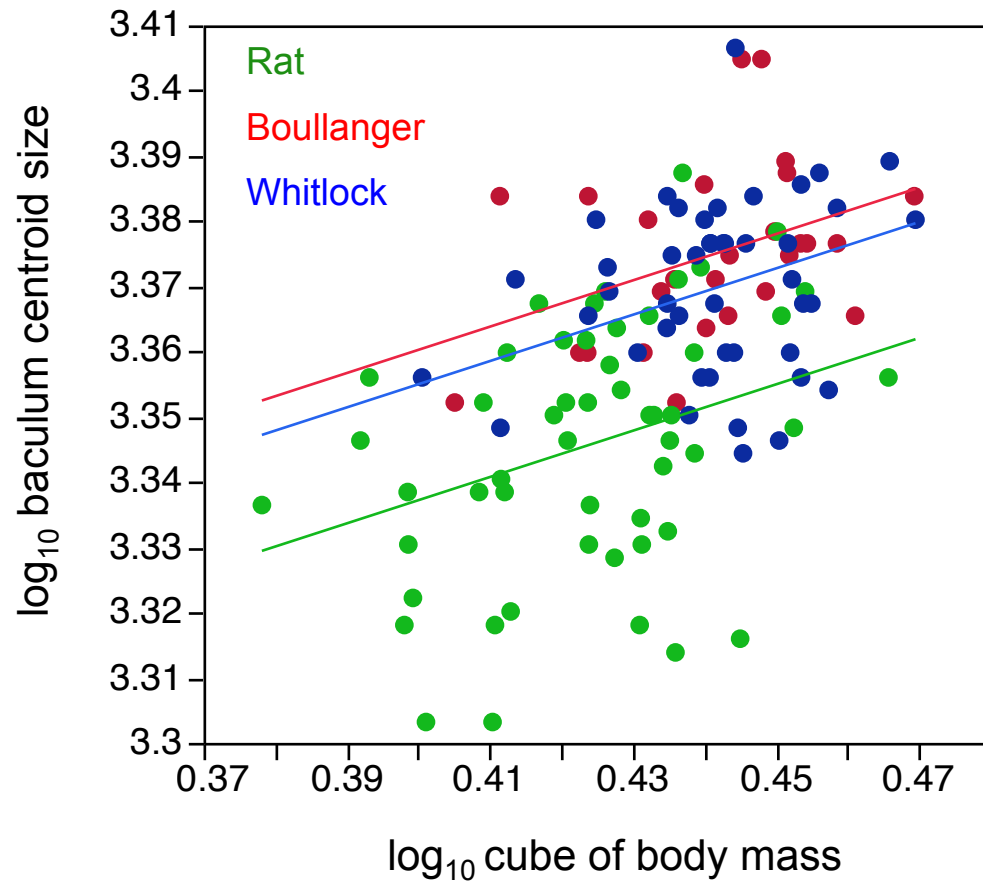


Figure 1

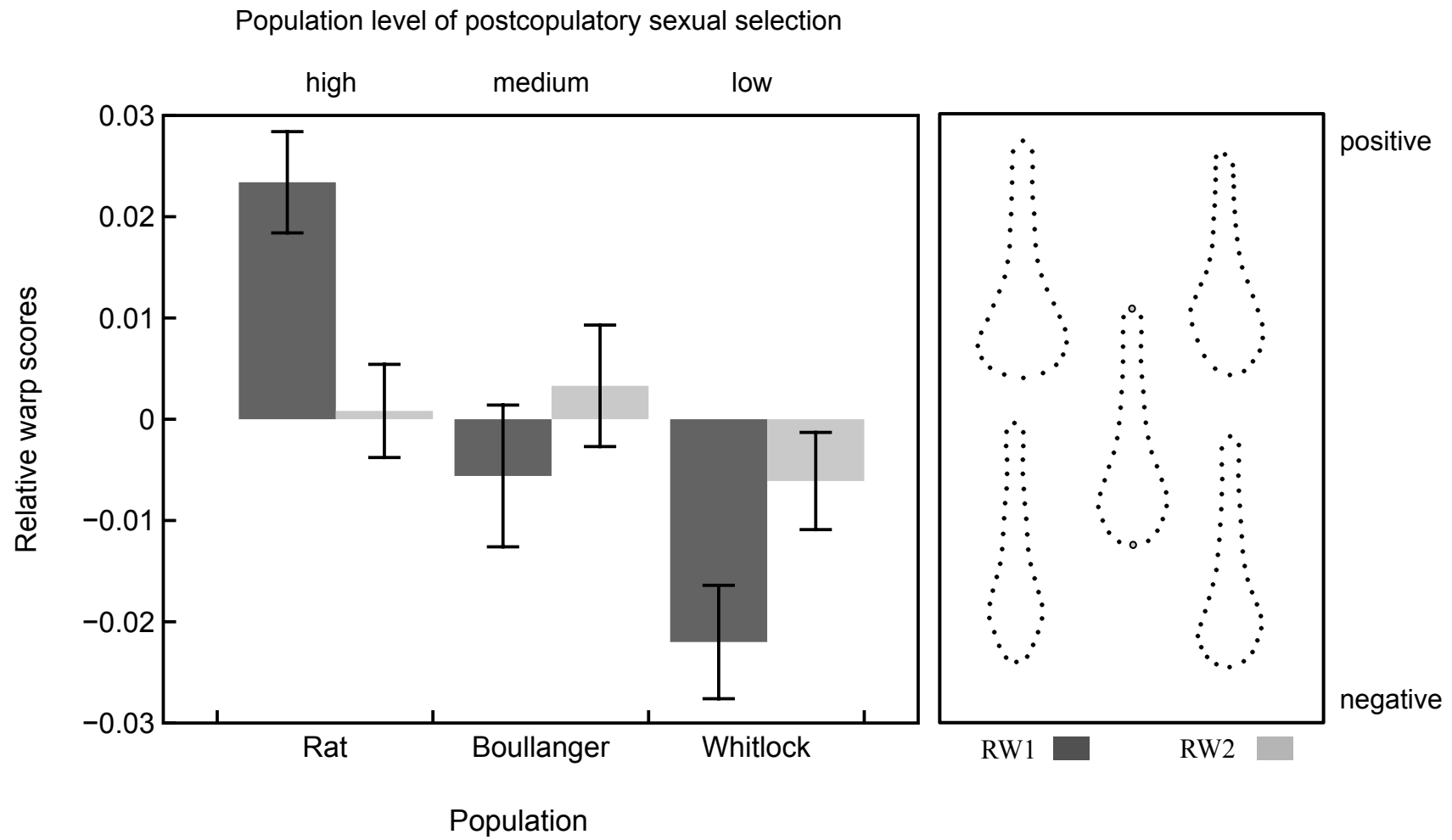


Figure 2

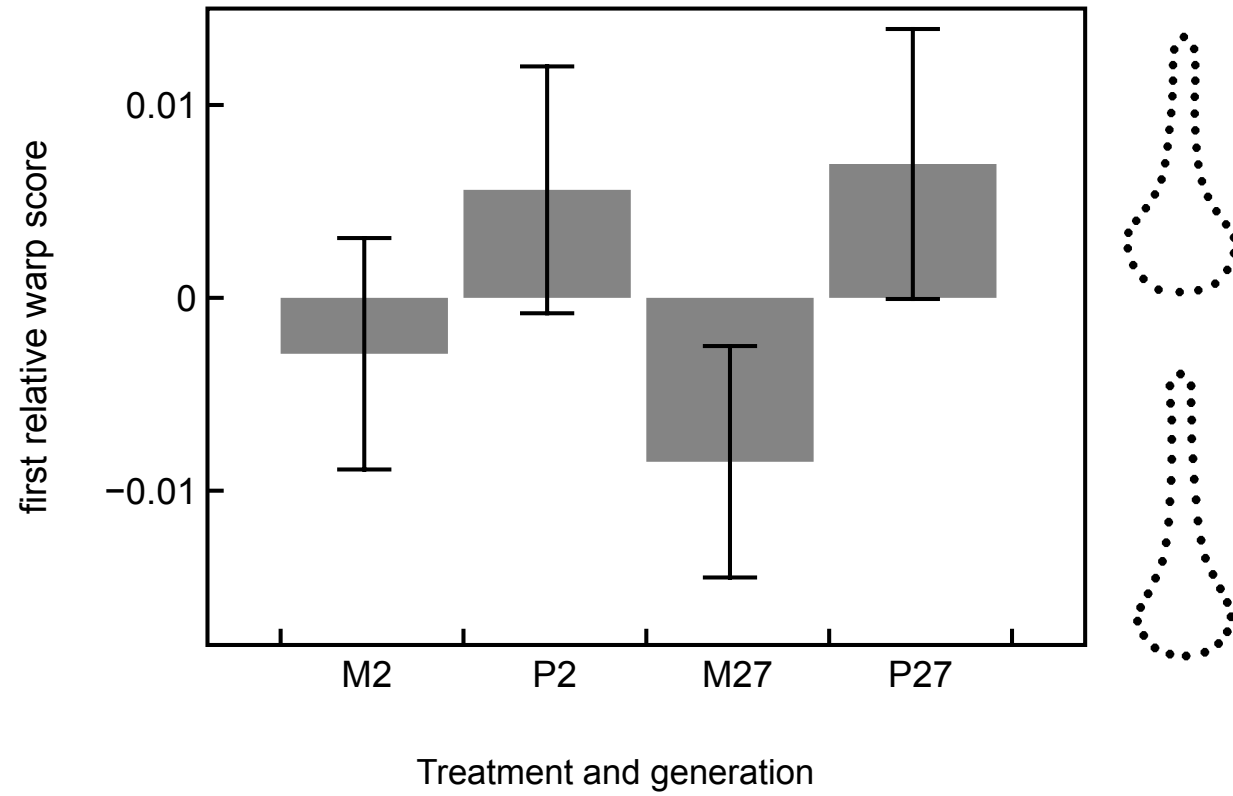


Figure 3