Research

Cite this article: André GI, Firman RC, Simmons LW. 2020 Baculum shape and paternity success in house mice: evidence for genital coevolution. *Phil. Trans. R. Soc. B* **375**: 20200150.
<http://dx.doi.org/10.1098/rstb.2020.0150>

Accepted: 16 July 2020

One contribution of 22 to a theme issue 'Fifty years of sperm competition'.

Subject Areas:
evolution

Keywords:
sperm competition, cryptic female choice, sexual conflict, os penis, sexual selection

Author for correspondence:

Goncalo I. André
e-mail: goncalo.igrejaandre@research.uwa.edu.au

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5106158>.

Baculum shape and paternity success in house mice: evidence for genital coevolution

Goncalo I. André, Renée C. Firman and Leigh W. Simmons

¹Centre for Evolutionary Biology, School of Biological Sciences (M092), The University of Western Australia, Crawley, 6009 Western Australia, Australia

ORCID GIA, 0000-0002-1513-4832; RCF, 0000-0001-9428-7388; LWS, 0000-0003-0562-1474

Sexual selection is believed to be responsible for the rapid divergence of male genitalia, which is a widely observed phenomenon across different taxa. Among mammals, the stimulatory role of male genitalia and female 'sensory perception' has been suggested to explain these evolutionary patterns. Recent research on house mice has shown that baculum (penis bone) shape can respond to experimentally imposed sexual selection. Here, we explore the adaptive value of baculum shape by performing two experiments that examine the effects of male and female genitalia on male reproductive success. Thus, we selected house mice (*Mus musculus domesticus*) from families characterized by extremes in baculum shape (relative width) and examined paternity success in both non-competitive (monogamous) and competitive (polyandrous) contexts. Our analyses revealed that the relative baculum shape of competing males influenced competitive paternity success, but that this effect was dependent on the breeding value for baculum shape of the family from which females were derived. Our data provide novel insight into the potential mechanisms underlying the evolution of the house mouse baculum and lend support to the stimulatory hypothesis for the coevolution of male and female genitalia.

This article is part of the theme issue 'Fifty years of sperm competition'.

1. Introduction

In sexually reproducing species with internal insemination, external male genitalia are essential for the deposition of the ejaculate into the female reproductive tract. Animal species in which females mate with multiple males are known to exhibit rapid divergent evolution in male genitalia [1,2], and there is now substantial evidence for the role of sexual selection in male genital evolution [1,3–5]. Three mechanisms of sexual selection have been proposed for the evolution and coevolution of male and female genital morphology; sperm competition, cryptic female choice and sexual conflict [3,5–8].

Sexual selection by cryptic female choice proposes that females increase their reproductive fitness by biasing paternity outcome towards males whose genital morphology deliver more effective stimulation [3,6–9]. Sexual conflict occurs when the reproductive interest of males and females are not aligned, instigating an arms race between male and female genitalia over the control of mating and fertilization [3,7,10,11]. Sexual conflict may arise in the context of sperm competition over fertilizations [4,9,10] and/or female resistance to mating [3]. Both cryptic female choice and sexual conflict predict the coevolution of male genital trait and female preference. Regardless of the precise mechanism, there is now a wealth of evidence that sexual selection can act on male genital morphology and influence the evolution of male genital traits involved with mating, insemination, fertilization and overall male reproductive success [12–19].

While the coevolution of male and female genitalia has been documented in a wide variety of taxa [9], studies of the coevolution of male and female genitalia in mammals are limited to a single study [20]. The interaction between male and female genitalia of four different marine species suggests that both cryptic female choice and sexual conflict may contribute to genital coevolution [20]. The mammalian ossified penis or baculum is a common feature of male genitalia across most mammalian orders [21–23]. In house mice (*Mus musculus domesticus*), it is now evident that the shape of the baculum plays an essential role in postmating sexual selection and has the potential to influence male fitness. For example, we recently discovered that the shape of the house mouse baculum harbours significant additive genetic variance and covariance with cervix width [24], and has been shown to respond to experimentally imposed sexual selection [25]. Interestingly, baculum shape also exhibits plasticity in response to the social environment, with an elevated risk of sperm competition leading to qualitatively similar changes in baculum shape to the evolutionary response seen under elevated sexual selection [26]. Moreover, house mouse baculum morphology has been shown to influence male reproductive success in free-ranging experimental populations; males with a relatively wider baculum were more successful at securing paternity under competitive scenarios [17].

As an ossified structure, the baculum may have evolved to provide rigidity to the penis and so provide stimulation to the female reproductive tract [27]. Female genital stimulation is important in mammals and is known to play a key role in priming the reproductive tract for embryo implantation by inducing neuroendocrine and physiological changes during and after copulation [28]. For mammal species, it is, therefore, reasonable to predict that female ability to discern variation in genital stimulation generates sexual selection on male genitalia [3,4,6,7]. Copulation in various mammals includes behaviours with the specific function of stimulating the female reproductive tract, many of which are required for a successful pregnancy [6,29]. Mammalian vaginal tracts are typically highly innervated [30,31], making them putative female preference traits that discern sensory stimulation from male genital traits.

In this study, we aimed to establish whether baculum size and shape have a causal effect on paternity success under both non-competitive and competitive mating scenarios, and to determine whether this effect is mediated by variation among females. We thus evaluated the effect of baculum morphology on embryo implantation rate and embryo viability in single mated females (monandrous matings), and the contribution of baculum size and shape to paternity success when two males compete for fertilization (polyandrous matings). We selected individuals from families known to lie at the extremes of genetic variation in baculum shape (herein referred to as ‘wide’ and ‘narrow’). We, therefore, had prior knowledge of the breeding values for genital morphology in our experimental pairings. Following the stimulation hypothesis [7,27,32], we expected that males with a relatively wide baculum would induce higher implantation rates and increased embryo viability compared to those males with a relatively narrow baculum. In polyandrous matings, we predicted that paternity success should depend on the shape of a male’s baculum relative to the shape of his rival’s baculum. Further, if sexual selection is responsible for the coevolution of male genital traits and female

preference traits [31], we predicted that the fitness effects of variation in baculum shape would depend on the breeding value for baculum morphology of the family from which females were derived.

2. Material and methods

(a) Source population and experimental animals

Male and female laboratory-reared house mice (*M. musculus domesticus*) were second and third-generation outbred descendants of wild individuals sourced from an isolated population on Rat Island located off the coast of Western Australia (28°42′ S, 113°47′ E). The source population was used to establish a paternal half-sibling breeding design to estimate patterns of additive genetic variation in baculum morphology, as described elsewhere [24]. Briefly, we selected a subset of F_1 offspring to conduct a morphometric analysis of baculum morphology from 30 paternal half-sibling families (90 maternal full-sibling families). The analysis revealed that all baculum shape measurements had moderate to high levels of additive genetic variation and heritability [24]. Baculum shape was described mainly by variation in the relative width of the baculum.

For the experiments described here, we selected one male and one female from the eight sire families at each extreme of baculum shape variation, for simplicity referred to as relatively narrow and relatively wide [24]. These mice were outbred under common garden conditions to generate families characterized by relatively narrow or wide bacula. Male and female pairs were housed together for a maximum of 14 days. When noted to be pregnant, females were separated and housed individually and received extra shredded paper as nesting material. At weaning age (21 days of age), female and male offspring were separated; female offspring were housed with their female siblings in standard mouse boxes (59 × 40 cm and 20 cm high) and male offspring were housed individually in smaller boxes (16 × 33 cm and 12 cm high). We used a sample of these offspring (F_2) for our first experiment that examined the effect of baculum shape on reproductive success in a non-competitive context (monogamous matings). The remaining offspring were used to generate grand-offspring (F_3) for our second experiment in which we examined the effect of baculum shape on competitive fertilization success (polyandrous matings). Baculum shape aligned with the expected baculum shape extremes in both the F_2 and F_3 generations (for details, see the electronic supplementary material, figures S1 and S2 and table S1). All animals were held in constant temperature rooms (CTR; 24°C) on a reverse dark–light cycle (10 : 14) and provided with water and food pellets (Speciality Feeds, WA) ad libitum. During pre-adult maturation, females were exposed once a week to unfamiliar and unrelated male odours, alternating between fresh male soiled chaff and fresh male urine. Females were virgin at the time of experiments, and males were sexually experienced by allowing them to mate with one unrelated female eight days prior to experimentation.

(b) Monandrous matings

Experimental matings ($n = 77$ mating pairs) were conducted when mice were approximately three months of age. At the onset of the dark phase, each female was inspected for oestrous condition by examining the appearance of the vaginal opening [33]. The oestrous cycle stage is based on variation in the colouration, striation, vaginal opening size and cell debris deposit described in detail elsewhere [24]. If in oestrous, females were paired with a male. Mating pairs were assigned according to baculum background extremes (see the electronic supplementary material, figure S3a). Mating sessions started approximately 5 h into the dark cycle when female sexual receptivity is at its peak [34]. Pairs were inspected for the presence of a mating plug every hour for evidence

of a complete ejaculatory series, and, therefore, successful mating [35]. Following mating, females were kept until mid-pregnancy (14 days of gestation) before being euthanized and dissected. The number ($n = 591$ embryos) and viability of the embryos, whether they were undergoing active development or being resorbed, were recorded ($n = 547$ viable embryos; $n = 44$ non-viable embryos) [36].

(c) Polyandrous matings

Mating procedures were the same as those described for monandrous matings except that females were mated consecutively with two males, one from each baculum background extreme ($n = 74$ mating trios, see the electronic supplementary material, figure S3b). Mating pairs were observed continuously for a maximum of 3 h or until ejaculation was observed. If unsuccessful, mating trials were stopped and male and female separated. Unsuccessful individuals were given three separate chances before being removed from the experiment. It has previously been shown that plug removal does not affect pregnancy initiation or litter size [37]. There is evidence, however, that the mating plug can influence the outcome of sperm competition [38,39]. The mating plug can delay second male ejaculation and effect second males' copulatory behaviour [38,39]. Because we were investigating the stimulatory role of the house mouse baculum and not its potential role in dislodging the mating plug, we removed the mating plug after each ejaculation. In this way, we ensured that each female received two ejaculations within their optimal receptive stage and that neither male was allowed to have its mating plug remain longer than 15 min. Thus, after a 15 min rest period, the first male's mating plug was removed from the female by gently pressing her against the side of the handling bin and dislodging it with a blunt probe. The female was then paired with the second male and again allowed to interact for a maximum of 3 h or until 15 min after receiving the second ejaculation. The second male's plug was removed as described above. Once matings were completed, females were placed in a clean box with nesting material, and euthanized and dissected after 14 days of gestation. Embryos were stored in 100% ethanol prior to paternity analysis as described below.

(d) Paternity assignment

For individuals used in the competitive matings, DNA was extracted for both parents and both viable and non-viable embryos (parents $n = 222$, embryos $n = 566$). DNA was extracted from the parental liver and embryonic head using the EDNA HISPEX extraction kit (Fisher Biotec, Subiaco, Western Australia, Australia). Paternity was assigned by screening 10 microsatellite loci (for more details, see the electronic supplementary material, table S2) [35,40]. Unlabelled primers were obtained from Integrated DNA Technologies (Singapore, Singapore). Labelled primers were obtained from Applied Biosystems (Foster City, CA, USA) (VIC) and Alpha DNA (Montreal, Canada) (FAM, PET and NED). Primers were divided into two multiplexes (electronic supplementary material, table S2) in 10 μ l reactions. Reactions contained 8 μ l of a multiplex kit (Qiagen, Doncaster, Victoria, Australia), 0.1 μ M of labelled forward primer mixed with 0.1 μ M of unlabelled forward primer, 0.2 μ M of unlabelled reverse primer and approximately 200 ng of template DNA. The thermocycling annealing temperature varied according to the multiplex; multiplex 1 annealing temperature was 60°C and multiplex 2 was 55°C. The rest of the thermocycling profile was identical: 5 min denature at 94°C, 30 cycles of 94°C for 30 s, 60°C/55°C for 30 s and 72°C for 1 min, followed by 72°C for 3 min. Polymerase chain reaction products (2.5 μ l) were run on an ABI3730 Sequencer, sized using Genescan-500 LIZ size standard and genotyped using GENEMAPPER software (v. 3.0; Life Technologies). Paternity assignment was assigned by manual exclusion.

(e) Morphometric analysis

Seven days after mating, males were euthanized, body weight was recorded and the baculum dissected for geometric morphometric analysis (GMA). Details of baculum dissection and morphometric analysis are described elsewhere [26]. Briefly, after baculum dissection, digital images were captured and GMA conducted using the software Tpsrelw 1.65 developed by Rohlf [41]. Landmarks were placed around the periphery of the baculum (36 sliding, four fixed) and relative warps (RWs) and centroid size were extracted (electronic supplementary material, figure S4). We focused on those RWs that individually explained more than 10% of the variation in shape and on centroid size. We assessed the reliability of landmark placement and repeatability of our measurements by placing landmarks on two images on two separate occasions for 20% of the analysed specimens for both generations. Our measurements of genital shape and size were highly repeatable (see the electronic supplementary material, table S3).

(f) Statistical analysis

All statistical analyses were performed in R, v. 3.6.1 (R Core Team 2015). For the non-competitive experiment, we explored the effect of baculum morphology and its interaction with female genetic background (wide/narrow) on the number of implanted embryos and embryo viability. To analyse embryo viability, we used generalized linear mixed models (GLMMs) with Laplace approximation and binomial error distributions. The number of viable embryos was included as the dependent variable and the total number of embryos as the binomial denominator. In our GLMM of embryo number, we used a Poisson error distribution. For all analyses, baculum size was rescaled by centring and dividing by two standard deviations. In some cases, more than one male and/or female had to be used from the same full-sibling family, so male and female family were modelled as random effects in the analyses.

In the polyandrous matings, we investigated whether the interaction between the first and second male baculum morphology and female genetic background affected the paternity share of the second male (P_2). We reduced the number of predictor variables by subtracting the first male RWs and centroid size from those of the second male, providing a measure of relative baculum shape and size for competing males. Because RW scores are centred to zero, we transformed them by adding a positive constant (10) to facilitate interpretation. P_2 was analysed by GLMM with Laplace approximation and binomial error distribution, and with the number of embryos sired by the second male included as the dependent variable and the total number of embryos as the binomial denominator. Both first and second male and female family were included as random effects.

3. Results

(a) Monandrous matings

The GMA returned two RWs that each explained more than 10% of the variation: RW1 = 44.58%; RW2 = 26.56%. RW1 described variation in the relative width and the extension of the baculum bulb along the shaft, while RW2 described variation in the shape and relative width of the baculum bulb (figure 1a). Baculum morphology and its interaction with female family background had no significant effect on the number of embryos (interaction terms—RW1: estimate = -0.275 ± 4.338 , $p = 0.95$; RW2: estimate = -5.975 ± 5.750 , $p = 0.29$; baculum size = -0.024 ± 0.171 , $p = 0.88$) or on their viability (interaction terms—RW1: estimate = -10.119 ± 17.112 , $p = 0.54$; RW2: estimate = 6.531 ± 21.766 , $p = 0.62$; baculum size = -0.272 ± 0.707 , $p = 0.44$). For full analyses, see the electronic supplementary material, tables S4 and S5.

Figure 1. Variation in baculum shape. (a) Among males in the monandrous experiment and (b) among males in the polyandrous experiment. Variation in baculum shape described by the extreme of RW 1 (1^- , 1^+), RW 2 (2^- , 2^+) and consensus shape (0). The positions of the 40 landmarks around the periphery of the baculum for both positive and negative extremes are shown.

Table 1. General linear mixed model (GLMM) of the effect of baculum shape of the first male relative to the second male and its interaction with the female's genetic background on second male parentage (males = 148; females = 74; total embryos = 519). (Values in italics are significant at $p < 0.05$.)

fixed effects	estimate $\pm$ (se)	type III, Wald χ^2	p	random effects	variance	$\pm$ s.d.
intercept	-0.565 (0.307)			1st male family	0.614	0.783
RW1	0.793 (7.619)	0.01	0.92	2nd male family	0.476	0.689
RW2	6.921 (7.543)	0.842	0.36	female family	<0.0001	<0.0001
baculum size	0.031 (1.784)	0.0003	0.98			
female extreme	0.493 (0.267)	3.404	0.06			
RW1 $\times$ female extreme	-7.252 (9.509)	0.582	0.44			
RW2 $\times$ female extreme	-23.26 (11.444)	4.133	<i>0.04</i>			
baculum size $\times$ female extreme	1.796 (2.818)	0.406	0.52			

(b) Polyandrous matings

The GMA again returned two RWs each explaining more than 10% of the variation in baculum shape: RW1 = 37.59%; RW2 = 29.80%. RW1 described variation in the relative width of the baculum bulb, while RW2 described variation in the relative width and the extension of the baculum bulb along the shaft (figure 1b).

Mating position (first or second male) had no effect on the proportion of embryos sired by the second male (Wilcoxon signed-rank test with continuity correction, $V = 1004.5$, $p = 0.11$; mean $P_2 = 0.45 \pm 0.15$). However, there was a significant interaction between the difference in RW2 between the first and second male and female genetic background on the proportion of offspring sired by the second male, P_2 (table 1). Thus, among females from families with a breeding value

for narrow bacula, P_2 decreased as the second male's baculum, relative to the first male, became wider (figure 2). Yet, among females from families with a breeding value for wide bacula, P_2 increased as the second male's baculum, relative to the first male, became wider (figure 2). In other words, among 'narrow' females, maximum P_2 ($47.12\% \pm 4.85\%$) was achieved with a relatively narrow baculum, and among 'wide' females, maximum P_2 ($41.79 \pm 5.46\%$) was achieved with a relatively wider baculum. RW1, baculum size and their interaction with female genetic background had no significant effects on second male parentage (table 1).

Owing to low variance at the microsatellite loci, paternity could only be assigned to 519 of the 566 embryos. To ensure that there was no bias introduced into our results from the loss of embryos to which paternity could not be assigned,

Figure 2. Effects of relative baculum width (RW2 first male–second male) and female genetic background on second male parentage. The plot shows the predict values (marginal effects) of the difference in baculum shape between the first and second male (1stRW2–2ndRW2) for males mating to females from a family with a breeding value for relatively wide bacula (red) and relatively narrow bacula (blue), with 95% confidence intervals. Negative values on the x-axis represent second males with a narrower baculum than the first male, and positive values represent second males with a wider baculum than the first male. A score of zero represents competing males with the same shaped baculum. Baculum shapes at the extremes of shape variation are shown for qualitative interpretation.

we conducted a statistical analysis excluding families in which more than 20% of the offspring could not be assigned and obtained quantitatively similar results to the full analysis (see the electronic supplementary material, table S6).

4. Discussion

Polyandry is a common mating strategy across many species [42–44], including the house mouse [45,46]. In natural mouse populations, the rate of multiple paternity can reach levels of up to 70%, indicating that post-copulatory male competition is intense [45,46]. In an earlier study on the source population used in these experiments, we discovered that 38% of females captured in the field had litters sired by two or more males [46]. Our previous research has also established that (i) baculum shape harbours significant additive genetic variation [24], (ii) can respond to the experimental manipulation of post-copulatory sexual selection [25], and (iii) males from the population that seeded this experiment had relatively wider bacula than males from populations with lower levels of multiple paternity [25]. Here, we found that baculum shape influenced male fitness when female house mice mated multiply, and thereby provide further evidence that baculum shape contributes to male reproductive success [17]. Moreover, our results show that the effect of baculum shape on male competitive fitness is dependent on the breeding value for baculum shape for the family from which females were derived. Collectively, these studies provide strong support for the role of post-copulatory selection in the coevolution of house mouse genitalia.

Our analyses revealed that the baculum shape did not affect male fitness when females mated monandrously. This finding provides evidence that it is the effect of the baculum on a male's ability to induce sperm transport and/or utilization that has the potential to contribute to male reproductive success, rather than its effect on embryo implantation and/or embryo survival. All males, regardless of the shape of their baculum, attained similar reproductive success when mating in a non-competitive context. However, our data offer support for the role of female sensory perception in imposing selection on the house mouse baculum in a competitive context. We found that when mated to two males, females from families characterized by males with a relatively wide baculum were increasingly likely to have offspring sired by second males as their baculum width relative to the first male increased, while females from families characterized by males with a relatively narrow baculum were increasingly likely to have offspring sired by second males as their baculum width relative to the first male decreased. Thus, paternity outcome in our experiments suggest a process of assortative fertilization with respect to genital traits. Taken in isolation, this finding could be interpreted as evidence for the coevolution of male and female genitalia under natural selection for morphological accommodation [3,5,7]. This seems unlikely given that baculum shape has been found to vary among populations in relation to the strength of post-copulatory sexual selection, and that baculum width increases in populations subjected to experimentally imposed post-copulatory sexual selection [25]. The interaction effects we observed are also compatible with coevolution of male and female traits via sexual selection mechanisms.

A Fisherian mechanism of cryptic female choice also predicts the establishment of genetic covariation between male genital trait and female preference [6,47]. The supposition is that females with the preference should bias paternity towards males with the preferred trait(s) so that genes for the preference and trait become linked [48,49]. The interaction effect on second male paternity could, therefore, be compatible with a mechanism of cryptic female choice based on the Fisher process if females from families characterized by wide or narrow bacula had preferences for wide or narrow bacula, respectively. The interaction effect could also reflect sexual conflict. A sensory response to copulation that promotes fertilization could favour males better equipped to stimulate females, to the extent that males exploit the female sensory responses to their own fitness gain [50]. If costly to females, antagonistic selection is expected to favour females that become resistant or less responsive to stimulation [50]. Recently, we showed that baculum shape covaries with the width of the genital cervix, consistent with the genetic coevolution of male and female genitalia predicted by models of sexual selection [24]. It may be, therefore, that females with a wide cervix are increasingly stimulated by second males as their baculum width increases, generating an increase in paternity share, but females with a narrow cervix become increasingly overstimulated with negative effects on paternity.

While certainly possible, it seems unlikely that cervix width alone can be responsible for the biasing of paternity in response to baculum shape that we have observed. Similar to other mammalian species, the neuronal arrangement of the house mouse vaginal tract comprises sympathetic, parasympathetic and sensory fibres [31], and is susceptible to regional, temporal and mating experience difference in innervation [30,31].

Variation in baculum width may lead to variation in the stimulation of individual nerve fibres and/or the overall stimulation that females receive and perceive during copulation. Females from different genetic backgrounds (narrow/wide) may favour males with different baculum widths owing to the type of neuronal fibres being stimulated as well as the number of, and the degree to which the neuronal fibres are stimulated. Further, females from families with breeding values for relatively narrow and wide bacula may vary in the distribution and/or quantity of nerve fibres surrounding the reproductive tract that may consequently lead to a differential perception of stimulation according to baculum width. Future studies should examine more closely variation in female genital tract innervation and sensory responses to variation in baculum shape.

In conclusion, our data show that baculum shape affects male competitive fertilization success when female house mice mate with more than one male. Our finding of an interaction between the difference in baculum shape between rival males and female genetic background provides a mechanism by which the male and female genitalia can coevolve in this species. Research on female tract innervation and variation in

the neuroendocrine response following copulatory stimulation are likely to bring a more nuanced understanding of how female genitalia as a preference trait impose selection for the evolution of male genital morphology. The research above would also bring an understanding of the relative importance of cryptic female choice and sexual sexually antagonistic coevolution in the evolution of mammalian genitalia.

Ethics. The work reported in this article followed all the guidelines for the ethical treatment of animals in research under UWA Ethics Committee approval (RA/3/100/1456).

Data accessibility. Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.9ghx3fff1> [51].

Authors' contributions. G.I.A., R.C.F. and L.W.S. designed the study. G.I.A. collected the source animals, generated and analysed the data and wrote the first draft of the manuscript. All authors contributed to the final version.

Competing interests. We declare we have no competing interests.

Funding. This research was funded by the Australian Research Council (grant no. LWS DP 170101315)

Acknowledgements. We thank E. Daymond for help with animal maintenance and monitoring animals during the mating trials. We thank M. Lovegrove and Y. Hitchen for help with the paternity assignment.

References

- Eberhard WG. 1985 *Sexual selection and animal genitalia*. Cambridge, MA: Harvard University Press.
- Arnqvist G. 1998 Comparative evidence for evolution of genitalia by sexual selection. *Nature* **393**, 784–786. (doi:10.1038/31689)
- Eberhard WG. 2010 Evolution of genitalia: theories, evidence, and new directions. *Genetica* **138**, 5–18. (doi:10.1007/s10709-009-9358-y)
- Hosken DJ, Stockley P. 2004 Sexual selection and genital evolution. *Trends Ecol. Evol.* **19**, 87–93. (doi:10.1016/j.tree.2003.11.012)
- Simmons LW. 2014 Sexual selection and genital evolution. *Austral. Entomol.* **53**, 1–17. (doi:10.1111/aen.12053)
- Eberhard WG. 1996 *Female control: sexual selection by cryptic female choice*. Princeton, NJ: Princeton University Press.
- Brennan PLR, Prum RO. 2015 Mechanisms and evidence of genital coevolution: the roles of natural selection, mate choice, and sexual conflict. *Cold Spring Harb. Perspect. Biol.* **7**, 1–21. (doi:10.1101/cshperspect.a017749)
- Firman RC, Gasparini C, Manier MK, Pizzari T. 2017 Postmating female control: 20 years of cryptic female choice. *Trends Ecol. Evol.* **32**, 368–382. (doi:10.1016/j.tree.2017.02.010)
- Sloan NS, Simmons LW. 2019 The evolution of female genitalia. *J. Evol. Biol.* **32**, 882–899. (doi:10.1111/jeb.13503)
- Chapman T, Arnqvist G, Bangham J, Rowe L. 2003 Sexual conflict. *Trends Ecol. Evol.* **18**, 41–47. (doi:10.1016/S0169-5347(02)00004-6)
- Parker GA. 2006 Sexual conflict over mating and fertilization: an overview. *Phil. Trans. R. Soc. B* **361**, 235–259. (doi:10.1098/rstb.2005.1785)
- Arnqvist G, Danielsson I. 1999 Copulatory behaviour, genital morphology, and male fertilization success in water striders. *Evolution (NY)* **53**, 147–156. (doi:10.1111/j.1558-5646.1999.tb05340.x)
- Danielsson I, Askenmo C. 1999 Male genital traits and mating interval affect male fertilization success in the water strider *Gerris lacustris*. *Behav. Ecol. Sociobiol.* **46**, 149–156. (doi:10.1007/s002650050604)
- House CM, Simmons LW. 2003 Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. *Proc. R. Soc. Lond. B* **270**, 447–455. (doi:10.1098/rspb.2002.2266)
- Hotzy C, Arnqvist G. 2009 Sperm competition favors harmful males in seed beetles. *Curr. Biol.* **19**, 404–407. (doi:10.1016/j.cub.2009.01.045)
- Wojcieszek JM, Simmons LW. 2011 Male genital morphology influences paternity success in the millipede *Antichiropus variabilis*. *Behav. Ecol. Sociobiol.* **65**, 1843–1856. (doi:10.1007/s00265-011-1192-4)
- Stockley P, Ramm SA, Sherborne AL, Thom MDF, Paterson S, Hurst JL. 2013 Baculum morphology predicts reproductive success of male house mice under sexual selection. *BMC Biol.* **11**, 66. (doi:10.1186/1741-7007-11-66)
- Briceno RD, Eberhard WG. 2009 Experimental modifications imply a stimulatory function for male tsetse fly genitalia, supporting cryptic female choice theory. *J. Evol. Biol.* **22**, 1516–1525. (doi:10.1111/j.1420-9101.2009.01761.x)
- Rodríguez V, Windson D, Eberhard W. 2004 *Experimental demonstrations of a sexually selected advantage for longer male genitalia in the tortoise beetle, Chelymophra alternans*. The Hague, The Netherlands: SBP Academic Publishing.
- Orbach DN, Kelly DA, Solano M, Brennan PLR. 2017 Genital interactions during simulated copulation among marine mammals. *Proc. R. Soc. B* **284**, 20171265. (doi:10.1098/rspb.2017.1265)
- Ramm SA. 2007 Sexual selection and genital evolution in mammals: a phylogenetic analysis of baculum length. *Am. Nat.* **169**, 360–369. (doi:10.1086/510688)
- Stockley P. 2012 The baculum. *Curr. Biol.* **22**, 1032–1033. (doi:10.1016/j.cub.2012.11.001)
- Brindle M, Opie C. 2016 Postcopulatory sexual selection influences baculum evolution in primates and carnivores. *Proc. R. Soc. B* **283**, 20161736. (doi:10.1098/rspb.2016.1736)
- André GI, Firman RC, Simmons LW. 2020 The coevolution of male and female genitalia in a mammal: a quantitative genetic insight. *Evolution (NY)* **74**, 1558–1567. (doi:10.1111/evo.14031)
- Simmons LW, Firman RC. 2014 Experimental evidence for the evolution of the mammalian baculum by sexual selection. *Evolution (NY)* **68**, 276–283. (doi:10.1111/evo.12229)
- André GI, Firman RC, Simmons LW. 2018 Phenotypic plasticity in genitalia: baculum shape responds to sperm competition risk in house mice. *Proc. R. Soc. B* **285**, 20181086. (doi:10.1098/rspb.2018.1086)
- Greenwald GS. 1956 The reproductive cycle of the field mouse, *Microtus californicus*. *J. Mammal.* **37**, 213. (doi:10.2307/1376680)
- Argiolas A, Melis MR. 2013 Neuropeptides and central control of sexual behaviour from the past to the present: a review. *Prog. Neurobiol.* **108**, 80–107. (doi:10.1016/j.pneurobio.2013.06.006)

29. Pavličev M, Wagner G. 2016 The evolutionary origin of female orgasm. *J. Exp. Zool. Part B Mol. Dev. Evol.* **326**, 326–337. (doi:10.1002/jez.b.22690)
30. Barry CM, Ji E, Sharma H, Beukes L, Vilimas PI, DeGraaf YC, Matusica D, Haberberger RV. 2017 Morphological and neurochemical differences in peptidergic nerve fibers of the mouse vagina. *J. Comp. Neurol.* **525**, 2394–2410. (doi:10.1002/cne.24214)
31. van Helden DF, Kamiya A, Kelsey S, Laver DR, Jobling P, Mitsui R, Hashitani H. 2017 Nerve-induced responses of mouse vaginal smooth muscle. *Pflugers Arch. Eur. J. Physiol.* **469**, 1373–1385. (doi:10.1007/s00424-017-1995-x)
32. Larivière S, Ferguson SH. 2002 On the evolution of the mammalian baculum: vaginal friction, prolonged intromission or induced ovulation? *Mamm. Rev.* **32**, 283–294. (doi:10.1046/j.1365-2907.2002.00112.x)
33. Byers SL, Wiles MV, Dunn SL, Taft RA. 2012 Mouse estrous cycle identification tool and images. *PLoS ONE* **7**, e35538. (doi:10.1371/journal.pone.0035538)
34. Gomendio M, Harcourt AH, Roldán ERS. 1998 Sperm competition in mammals. In *Sperm competition and sexual selection* (eds T Birkhead, AP Møller), pp. 667–755. Amsterdam, The Netherlands: Elsevier.
35. Dietrich W, Katz H, Lincoln SE, Shin HS, Friedman J, Dracopoli NC, Lander ES. 1992 A genetic map of the mouse suitable for typing intraspecific crosses. *Genetics* **131**, 423–447.
36. Firman RC, Simmons LW. 2012 Male house mice evolving with post-copulatory sexual selection sire embryos with increased viability. *Ecol. Lett.* **15**, 42–46. (doi:10.1111/j.1461-0248.2011.01706.x)
37. Firman RC, Simmons LW. 2010 Experimental evolution of sperm quality via postcopulatory sexual selection in house mice. *Evolution (NY)* **64**, 1245–1256. (doi:10.1111/j.1558-5646.2009.00894.x)
38. Sutter A, Lindholm AK. 2016 The copulatory plug delays ejaculation by rival males and affects sperm competition outcome in house mice. *J. Evol. Biol.* **29**, 1617–1630. (doi:10.1111/jeb.12898)
39. Sutter A, Simmons LW, Lindholm AK, Firman RC. 2016 Function of copulatory plugs in house mice: mating behavior and paternity outcomes of rival males. *Behav. Ecol.* **27**, 185–195. (doi:10.1093/beheco/arv138)
40. Sutter A. 2015 Detrimental effects of an autosomal selfish genetic element on sperm competitiveness in house mice. *Proc. R. Soc. B* **282**, 20150974. (doi:10.1098/rspb.2015.0974)
41. Rohlf FJ. 2006 *tpsDig, digitize landmarks and outlines, v.2.05*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.
42. Birkhead TR. 1998 Cryptic female choice: criteria for establishing female sperm choice. *Evolution (NY)* **52**, 1212. (doi:10.2307/2411251)
43. Simmons LW. 2001 *Sperm competition and its evolutionary consequences in the insects*. Princeton, NJ: Princeton University Press. See <https://books.google.com.au/books?id=iuQ9DwAAQBAJ>.
44. Bretman A, Tregenza T. 2005 Measuring polyandry in wild populations: a case study using promiscuous crickets. *Mol. Ecol.* **14**, 2169–2179. (doi:10.1111/j.1365-294X.2005.02556.x)
45. Dean MD, Ardlie KG, Nachman MW. 2006 The frequency of multiple paternity suggests that sperm competition is common in house mice (*Mus domesticus*). *Mol. Ecol.* **15**, 4141–4151. (doi:10.1111/j.1365-294X.2006.03068.x)
46. Firman RC, Simmons LW. 2008 The frequency of multiple paternity predicts variation in testes size among island populations of house mice. *J. Evol. Biol.* **21**, 1524–1533. (doi:10.1111/j.1420-9101.2008.01612.x)
47. Mead LS, Arnold SJ. 2004 Quantitative genetic models of sexual selection. *Trends Ecol. Evol.* **19**, 264–271. (doi:10.1016/j.tree.2004.03.003)
48. Prum RO. 2010 The Lande-Kirkpatrick mechanism is the null model of evolution by intersexual selection: implications for meaning, honesty, and design in intersexual signals. *Evolution (NY)* **64**, 3085–3100. (doi:10.1111/j.1558-5646.2010.01054.x)
49. Greenfield MD, Alem S, Limousin D, Bailey NW. 2014 The dilemma of Fisherian sexual selection: mate choice for indirect benefits despite rarity and overall weakness of trait-preference genetic correlation. *Evolution (NY)* **68**, 3524–3536. (doi:10.1111/evo.12542)
50. Arnqvist G. 2006 Sensory exploitation and sexual conflict. *Phil. Trans. R. Soc. B* **361**, 375–386. (doi:10.1098/rstb.2005.1790)
51. André GI, Firman RC, Simmons LW. 2020 Data from: Baculum shape and paternity success in house mice: evidence for genital coevolution. Dryad Digital Repository. (<https://doi.org/10.5061/dryad.9ghx3fff1>)