



SYMPOSIUM

The Baculum was Gained and Lost Multiple Times during Mammalian Evolution

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Synopsis The rapid evolution of male genitalia is a nearly ubiquitous pattern across sexually reproducing organisms, likely driven by the evolutionary pressures of male–male competition, male–female interactions, and perhaps pleiotropic effects of selection. The penis of many mammalian species contains a baculum, a bone that displays astonishing morphological diversity. The evolution of baculum size and shape does not consistently correlate with any aspects of mating system, hindering our understanding of the evolutionary processes affecting it. One potential explanation for the lack of consistent comparative results is that the baculum is not actually a homologous structure. If the baculum of different groups evolved independently, then the assumption of homology inherent in comparative studies is violated. Here, we specifically test this hypothesis by modeling the presence/absence of bacula of 954 mammalian species across a well-established phylogeny and show that the baculum evolved a minimum of nine times, and was lost a minimum of ten times. Three different forms of bootstrapping show our results are robust to species sampling. Furthermore, groups with a baculum show evidence of higher rates of diversification. Our study offers an explanation for the inconsistent results in the literature, and provides insight into the evolution of this remarkable structure.

Introduction

Characterizing the evolutionary forces that drive rapid divergence of morphological structures is fundamental to understanding adaptation, speciation, and the diversity of life. Across nearly all sexually reproducing organisms, male genital anatomy evolves more rapidly than other morphological structures (Eberhard 1985; Romer and Parsons 1986; Klaczko et al. 2015). In fact, male genitals diverge so rapidly that taxonomists often use them to distinguish closely related species that are otherwise morphologically indistinguishable (Wade and Gilbert 1940; Hamilton 1949; Adams and Sutton 1968; Patterson and Thaler 1982; Simson et al. 1993).

The baculum is a bone that occurs in the penis of many mammal species, and they display astonishing morphological diversity (Chaine 1925; Eadie 1947; Burt 1960; Romer and Parsons 1986; Dixson 1995;

Weimann et al. 2014). Qualitatively, interspecific divergence exceeds intraspecific polymorphism, a classic signature that suggests the baculum is a target of recurrent adaptive evolution.

Several hypotheses for the function of the baculum have been proposed which lead to testable predictions in a comparative framework. Unfortunately, comparative studies have failed to yield general and consistent results (summarized in Table 1). One hypothesis is that the baculum protects the urethra and provides mechanical support during copulation (Long and Frank 1968; Oosthuizen and Miller 2000; Dyck et al. 2004). Dixson found that in pinipeds and primates, species with prolonged intromission tended to have more elongate bacula (Dixson 1987a, 1987b, 1995, 1998). However, across 52 species of carnivores, baculum length did

Table 1 Hypothesized functions for the baculum, their predictions, and their support (or not) among comparative studies

Hypothesized function	Prediction	Prediction supported	Not supported
Protect the urethra during copulation	Species with prolonged intromission should have longer bacula	Primates ^{a,b} , Carnivores ^c	Carnivores ^d
Functions in the context of sperm competition	Baculum length positively covaries with inferred mating system	Rodents ^e , Carnivores ^e	Bats ^{e,f} , Primates ^e
	Baculum length negatively covaries with sexual size dimorphism	Pinnipeds ^g	Carnivores ^d
	Baculum morphology predicts paternity under competitive conditions	Mice ^{h,i}	—
Stimulates female to ovulate or implant	Species with induced ovulation have longer bacula	—	Carnivores ^{c,d}
Signals male quality	Baculum displays positive allometry	Muskrats ^j , Seals ^{k,l}	Martens ^m , Mice ⁿ , Bats ^o

^aDixson (1987a); ^bDixson (1987b); ^cDixson (1995); ^dLarivière and Ferguson (2002); ^eRamm (2007); ^fHosken et al. (2001); ^gFitzpatrick et al. (2012); ^hStockley et al. (2013); ⁱSimmons and Firman (2014); ^jTasikas et al. (2009); ^kMiller and Burton (2001); ^lMiller et al. (1999); ^mSchulte-Hostedde et al. (2011); ⁿRamm et al. (2010); ^oLüpold et al. (2004).

not covary with intromission duration (Larivière and Ferguson 2002). Although Dixson (1995) concluded that carnivores with increased intromission length had longer bacula, that study did not incorporate modern methods to account for phylogenetic relationships.

Another set of hypotheses maintain the baculum functions in different aspects of the mating system, for example, by stimulating the female in a way that biases paternity toward a particular male (male–female interactions), assisting in the removal of sperm from prior males (sperm competition to increase “offensive” strategies of males), or by inducing damage to the female to inhibit remating (sexual conflict to increase “defensive” strategies of males). These latter two hypotheses predict a correlation between baculum morphology and the inferred risk or intensity of sperm competition. In an experimental evolution study within a single species of mouse, males evolved relatively wider bacula when subjected to a (Simmons and Firman 2014), lending support to the hypothesis that bacula function in the context of sperm competition. Across rodents and carnivores, baculum length increased with the inferred intensity of sperm competition (Ramm 2007), but no such correlation was found in bats or primates (Hosken et al. 2001; Ramm 2007). A corollary prediction is that baculum length negatively covaries with the degree of sexual dimorphism, since very strong dimorphism often indicates that males are investing disproportionately in precopulatory rather than postcopulatory competition (Parker et al. 2013; Lüpold et al. 2014; Dines et al. 2015). In some pinnipeds, there was a negative correlation between sexual size dimorphism and baculum size (Fitzpatrick et al. 2012), but this pattern was not observed across other carnivores (Larivière and Ferguson 2002).

If large bacula somehow indicate male quality (Miller and Burton 2001; Lüpold et al. 2004), then relatively fit males should invest disproportionately in ever larger bacula, and a positive allometric relationship should arise (but see Bonduriansky 2007). The relationships between baculum size and body size are inconsistent, ranging from positive allometry (Miller et al. 1999; Miller and Burton 2001; Tasikas et al. 2009), to isometry or even negative allometry (Lüpold et al. 2004; Ramm et al. 2010; Schulte-Hostedde et al. 2011).

In sum, there are no consistent relationships between features of the baculum and organismal biology (Table 1), hindering our understanding of the evolutionary forces affecting their morphological diversity. There are at least five potential explanations to reconcile inconsistencies from the literature. First, nearly all studies in Table 1 focus on the length of the baculum, and shape may be a more important parameter to test some of these hypotheses (Baryshnikov et al. 2003; Stockley et al. 2013). Unfortunately, modern morphometric techniques have only recently begun being applied to studies of baculum morphology (Schultz et al. forthcoming 2016). Second, the baculum may function in distinct biological processes across species, so that correlations to one group’s biology need not apply to another’s (Patterson and Thaler 1982; Kelly 2000). Third, the baculum may evolve so rapidly that it outpaces evolutionary correlation to other characters. Fourth, the baculum may function in different contexts across species, as evidenced by heterogeneity in its morphological placement. For example, in some groups the baculum is at the distal extreme of the glans, while in others it is more deeply embedded proximally (Patterson 1983).

A fifth hypothesis, and the main topic of the current study, is that the baculum evolved more than

once. If the baculum has similarly evolved multiple times, then the baculum should not be considered a homologous structure, in that it was not inherited through common descent with modification from a mammalian ancestor. Instead, multiple derivations would suggest that the baculum evolved in different biological contexts, possibly to solve different evolutionary challenges, confounding any straightforward correlations to aspects of organismal biology. Most importantly, multiple derivations would violate the fundamental assumption of homology that is inherent in any comparative analysis. Through intensive literature review and phylogenetic analysis, we provide strong evidence that the baculum has been gained and lost multiple times and groups that evolved a baculum appear to have diversified more rapidly than groups without. Our study helps explain inconsistencies observed in the literature and provides valuable insight into the evolution of this astonishing structure.

Materials and methods

Any phylogenetic analysis will be sensitive to the exact taxa sampled. For example, non-randomly including more species with bacula would bias the estimated rate of baculum gain upwards, since more evolutionary time would be spent with a baculum. To avoid such biases, we included as many species as possible in a large mammalian phylogeny, without *a priori* knowledge of their baculum status. It is possible that all previously published phylogenies are inherently biased toward including species with a baculum, since taxonomists often use this structure to delineate species when other morphologies fail to distinguish them. However, we expect such a bias to add relatively short external branches—for example, to separate extremely closely related species—and, therefore, would not compromise analyses over deeper evolutionary time. After describing our analytical pipeline, we introduce three different bootstrapping strategies to account for potential taxonomic and phylogenetic bias.

Phylogeny

Six phylogenies were merged to create a tree of 3707 mammal species (Supplementary File 1). The first, from Meredith et al. (2011), was a family-level phylogeny across mammals and served as the scaffold to which five more taxonomically focused studies were added. Meredith et al. (2011) used a likelihood framework to analyze a supermatrix that included 164 mammal species plus 5 outgroups, taken from 26 gene fragments consisting of 35,603 base pairs (bp)

and 11,010 amino acids. Although 164 species is a small fraction of the over 5000 mammalian species (Nowak 1999; Wilson and Reeder 2005), they included at least one representative from nearly every mammalian family, providing a reasonable foundation for phylogenetic inference. We then replaced specific nodes of the Meredith et al. (2011) phylogeny with larger phylogenies described below, in all cases normalizing branching times to the Meredith et al. (2011) scale, with one specific example given for the inclusion of the bat phylogeny, as described next.

Shi and Rabosky (2015) used a likelihood framework to analyze a supermatrix that included 812 bat species, gathered from 29 loci of 20,376 bp. All 20 bat families were represented by at least one species. We replaced the single bat clade from the Meredith et al. (2011) phylogeny with the single clade of 812 bat species from Shi and Rabosky (2015). In the Shi and Rabosky (2015) phylogeny, the most recent common ancestor of bats occurred at 115.3 units of genetic divergence. In the Meredith et al. (2011) phylogeny, the most recent common ancestor of bats occurred 131.1 million years ago. Therefore, we multiplied all branch lengths of Shi and Rabosky (2015) by 131.1/115.3 prior to its insertion into the Meredith et al. (2011) phylogeny, allowing us to maintain the branch scaling of the latter and preserve an ultrametric tree. Additional phylogenies discussed below were also normalized in the same manner.

Fabre et al. (2012) used a maximum likelihood framework to analyze a supermatrix that included 1265 rodent species, which represents more than 80% of known generic diversity in rodents, utilizing 11 loci. The Fabre et al. (2012) phylogeny replaced the rodent clade in Meredith et al. (2011).

McGowen et al. (2009) developed a molecular phylogeny through a Markov Chain Monte Carlo Bayesian analysis using 45 nuclear loci, transposons, and mitochondrial genomes from 87 Cetacean species. The McGowen et al. (2009) phylogeny replaced the Cetacean clade in Meredith et al. (2011).

Nyakatura and Bininda-Emonds (2012) built a supertree of 286 carnivore species using matrix representation parsimony from existing phylogenetic hypotheses and molecular data. The dataset included 114 phylogenetic hypotheses as well as 74 novel trees derived from 45,000 bp of sequence data. This phylogeny replaced the carnivore clade in Meredith et al. (2011).

Perelman et al. (2011) amplified 34,927 bp sequenced from 54 homologous genomic regions of primate species representing 186 species, then built a phylogeny using maximum likelihood. This primate phylogeny was the only one of the additional

five that was not published as ultrametric. We, therefore, converted this phylogeny to an ultrametric tree using the `CHRONOS` function in the R package `APE` (Paradis et al. 2004; Paradis 2012), using the eight fossil calibration dates provided in the legend of Fig. 1 of Perelman et al. (2011).

The overall combined phylogeny (Supplementary File 1) contained 3707 species, and was normalized to the scale of Meredith et al. (2011) to make it ultrametric. Uncertainty in the branching patterns was not considered, but any uncertainty is unlikely to greatly alter our main conclusions. The reason is that bacula arise either in localized groups of related species (or single lineages) or at deeper nodes that unite species at approximately the family level (Fig. 1). It is difficult to envision how minor branch swapping could affect our main conclusion, which is that the baculum evolved multiple times.

Baculum status

Through literature searching, we were able to score the presence/absence of the baculum in 1028 species (925 with a baculum, 103 without) (Supplementary Table 1). Most of these were represented in the phylogeny we created above so that patterns of evolution could be evaluated. The primary sources were the Index for Mammalian Species (Hayssen 2014), and Asdell's Patterns of Mammalian Reproduction (Asdell and Hubbs 1964). Additional data came from searches in Google Scholar (www.scholar.google.com), with the phrases baculum, bacula, os penis, os priapi (Latin, penis bone), l'os pénien (French, penis bone), penisknochen (German, penis bone), báculo (Portuguese, baculum), and various species names, museum databases iDigBio (www.idigbio.org) and Morphobank (www.morphobank.com), as well as personal communication with taxonomic experts. All primary sources are listed in Supplementary Table 1.

Any mention of ossification in the penis was considered a baculum, however sources were used only if they included images or measurements of bacula, and listed the exact species names. In some cases, the closest taxonomic level was used. For example, *Cratogeomys castanops* was included in the phylogeny of Fabre et al. (2012), but we could only find information on baculum presence for one of its congeners, *Cratogeomys merriami* (Burt 1960), which was not part of the rodent phylogeny. In this case, we scored *C. castanops* as having a baculum. Methodologically, this makes one of two assumptions: (1) species within the same genus share baculum state or (2) species from the same genus can be

swapped out in the phylogeny (in this case, we could have replaced *C. castanops* in the phylogeny with *C. merriami*). Of the 1028 species scored for baculum presence, 993 had species-level evidence (i.e., species names matched in both the phylogeny and the baculum literature), and 35 had genus-level evidence (e.g., the *Cratogeomys* example just discussed). We re-ran our analyses below after excluding the 35 species with genus-level evidence.

The literature is rife with claims of baculum presence/absence without data, which we excluded here. For example, it is often stated that no cetaceans have a baculum, when in fact only a few cetacean studies specifically report on the baculum (Supplementary Table 1). Similarly, two studies that simply mention the possibility that moon rats (*Podogymnura*) have a baculum (Kaudern 1907; Gerhardt 1909) are commonly mis-cited as providing evidence the baculum exists.

Testing evolutionary hypotheses

There was an overlap of 954 taxa between the 3707 species in the phylogeny and the 1028 species scored for baculum presence/absence. With these 954 species, we estimated the number of times bacula have been independently gained and lost using stochastic mapping as implemented in the function `MAKE.SIMMAP` of the R package `PHYTOOLS` (Revell 2012). Given an observed phylogenetic tree and distribution of character states, stochastic mapping generates multiple iterations of character evolution that are consistent with the observed character states, using a continuous time-reversible Markov model.

There are two main stages in stochastic mapping (Nielsen 2002; Huelsenbeck et al. 2003; Bollback 2006). First, the probabilities of possible ancestral states at all interior nodes are calculated (Felsenstein 1981). A collection of ancestral states is then sampled according to their state probabilities at each node. Every branch then starts at state i and ends at state j , with character states at the tips simply the observed baculum presence/absence for each species.

Second, potential character transitions are placed over each branch. In short, stochastic mapping produces randomly sampled character state histories that are consistent with the states at the tips of the tree by estimating transition rates and sampling ancestral states at internal nodes. We summarized baculum gains and losses from 1000 such histories. Visual representations were made using the `DENSITYMAP` function of `PHYTOOLS` (Revell 2012). We considered branches where at least 50% of the iterations

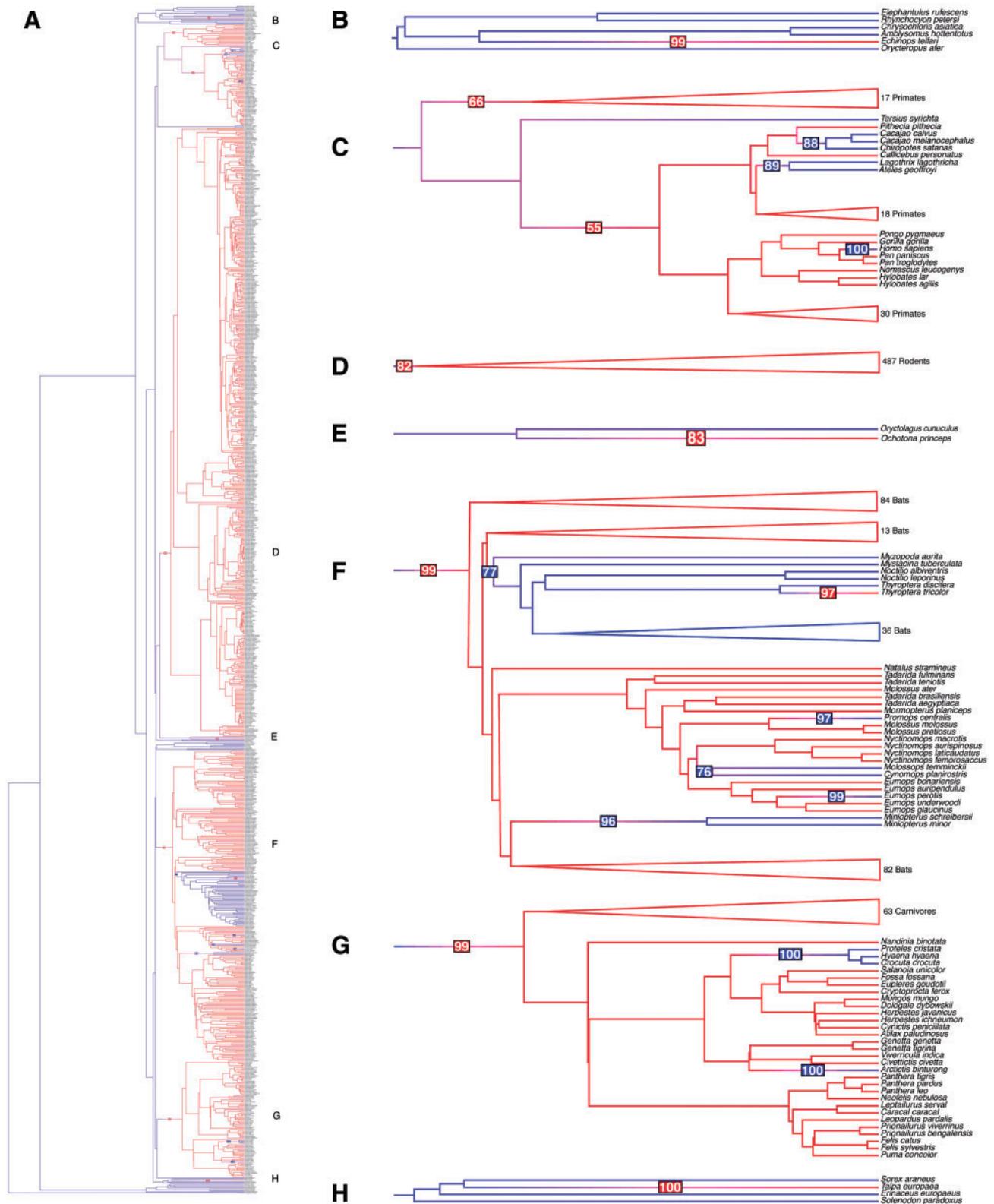


Fig. 1 (A) High confidence gains and losses of the baculum derived from stochastic mapping. Red branches indicate species with a baculum, blue without; numbers in red (blue) boxes indicate the proportion of iterations that mapped a baculum gain (loss) to those particular branches. Branches where a transition occurred in at least 50% of the iterations are highlighted in (B)–(H), which correspond to regions labeled B–H in panel A. Fig. 1A is meant to give an overall impression of the distribution of bacula across mammals; a zoomable version is provided as Supplementary Figure 1.

showed a gain or loss as “high confidence.” Since any cutoff is admittedly arbitrary, we also present the number of gains and losses observed in at least 95% of the iterations.

Stochastic mapping has a number of advantages over more traditional parsimony methods. Parsimony underestimates both the mean and variance of the number of character state changes because it allows at most a single transition along a branch for characters with only two states (Bollback 2006). With stochastic mapping, characters are allowed to change multiple times along a single branch, which allows uncertainty in the exact reconstruction of ancestral states to be accounted for (Nielsen 2002). Nevertheless, for comparative purposes we also evaluated transitions in a strict parsimony framework, using the `ANCESTRAL.PARS` function in the R package `PHANGORN` (Schliep 2011).

Bootstrapping

To evaluate the robustness of our results, we repeated the stochastic mapping procedure under three different bootstrapping regimes, all written with customized scripts in R (available from the authors at request). Under each regime, we subsampled 50%, 60%, 70%, 80%, or 90% of the species and repeated the stochastic mapping procedure 100 times each.

The first and most straightforward version of bootstrapping is to subsample species uniformly, with each species in the dataset equally likely of being excluded from any one bootstrap iteration. We refer to this first version as “uniform bootstrapping.” Although computationally easy to implement, this version of bootstrapping ignores potential taxonomic and phylogenetic biases, which the next two versions of bootstrapping address.

Second, to address potential taxonomic bias, we repeated the bootstrap, but species from mammalian clades that were sampled more (relative to the number of known extant species) had higher probabilities of being excluded during any one bootstrap iteration. For example, 95 of 294 (32%) known carnivore species, and 4 of 452 (0.009%) of known Eulipotyphla species, could be included in our current dataset (Supplementary Table 2). Therefore, carnivore species would be $32/0.009 = 4000$ times more likely to be excluded in any one bootstrap replicate compared to Eulipotyphla species. The probabilities that species from various groups were excluded per bootstrap replicate are given in Supplementary Table 2. We refer to this second version as “proportional bootstrapping.”

Third, to account for potential phylogenetic bias, we weighted each bootstrap replicate according to the average relationship of each species to all other species in the phylogeny, calculated using the `COPHENETIC` function in the R package `APE` (Paradis et al. 2004; Paradis 2012). Species that were more closely related to other species on the phylogeny had a higher probability of being excluded from bootstrap replicates, in an attempt to more evenly sample the mammal phylogeny. For example, the sister group *Oryzomys rostratus* + *Oryzomys melanotis* was separated by a cophenetic distance of 10^{-5} , while the sister group *Talpa europaea* + *Sorex araneus* was separated by 1.33 (with cophenetic distance in units of 100 million years). In this example, one of the *Oryzomys* species would be $1.33/10^{-5} = 13,000$ times more likely of being excluded in a bootstrap iteration. This bootstrap had to be recursive, where we excluded one species at a time, then re-evaluated the cophenetic matrix for the remaining ($n-1$) species before excluding the next, until the appropriate number of species was dropped. We refer to this third version as “cophenetic bootstrapping.”

Correlating baculum presence to rates of diversification

We tested whether groups with or without a baculum differed in estimated rates of diversification using methods of Binary State Speciation and Extinction (BiSSE). BiSSE was implemented using the R package `DIVERSITREE`, by creating a likelihood function with the `MAKE.BISSE` function (FitzJohn 2012). This function takes on six parameters: the speciation and extinction rates in groups with versus without a baculum, plus the transition rates from baculum absent \rightarrow present and present \rightarrow absent. Initial starting points for these six parameters and the necessary likelihood function were estimated using the `STARTING.POINT.BISSE` function, and the likelihood of the model estimated using the `FIND.MLE` function (FitzJohn 2012). The null model constrained the two speciation rates to be equal, using the `CONSTRAIN` function, then a likelihood ratio test (LRT) performed to infer whether the two speciation rates were significantly different from each other (FitzJohn 2012).

Results

The baculum was gained and lost multiple times

Across 1000 iterations of stochastic mapping, the baculum evolved an average of 9.5 times and was lost an average of 11.5 times (Fig. 1, Supplementary Fig. 1). Gains and losses clustered along 19 branches in

the phylogeny (Fig. 1, Supplementary Fig. 1). Specifically, 9 branches gained a baculum, and 10 branches lost a baculum in at least 50% of the 1000 iterations of stochastic mapping, with 5 gains and 6 losses occurring in at least 95% of the iterations. We thus conclude that the baculum evolved a minimum of 9 times and was lost a minimum of 10 times throughout mammalian evolution. We now discuss these 19 gains and losses in more depth, in general from “top to bottom” of the phylogeny (Fig. 1, Supplementary Fig. 1).

The baculum is only found among Eutherian mammals, and absent in basal species of Metatherians. Therefore, the ancestor of mammals lacked a baculum. Four baculum gains occurred in single lineages on our phylogeny—the hedgehog tenrec (*Echinops telfairi*, 99% of iterations, Fig. 1B), the American pika (*Ochotona princeps*, 83%, Fig. 1E), Spix’s disc-winged bat (*Thyroptera tricolor*, 97%, Fig. 1F), and the European mole (*Talpa europaea*, 100%, Fig. 1H).

In primates, the ancestral state remains uncertain with our data, but there were at least two independent gains, one in lemur-like primates (Strepsirhini, 66%) and another leading to a subset of monkeys and apes (Simiiformes, 55%) (Fig. 1C). The latter gain was followed by three independent losses, one in the *Cacajao* + *Chiropotes* clade (88%), one in the *Lagothrix* + *Ateles* clade (89%), and one in humans (*Homo sapiens*, 100%) (Fig. 1C). We are unaware of any scientific publication to suggest that extinct *Homo* species, including *H. neanderthalensis*, had a baculum. The lack of a baculum in *Tarsius syrichta* (Fig. 1C) was not strongly resolved to a loss in that lineage given the uncertainty in transitions at the base of the primate clade.

The common ancestor of all rodents gained a baculum (82%), followed by the maintenance of a baculum in all rodent species that we could include here (Fig. 1D). This was somewhat surprising, and implies that the incredible morphological diversity of rodent bacula (Burt 1960) occurs against a backdrop of evolutionary constraint maintaining the structure.

Bats showed somewhat complicated patterns (Fig. 1F). The baculum was gained in the common ancestor of all bats (99%), followed by five independent losses in (1) the ancestor of the 42-species clade that includes *Myzopoda aurita* (77%), (2) the big-crested mastiff bat (*Promops centralis*, 97%), (3) the dwarf dog-faced bat + southern dog-faced bat (*Molossops temminckii* + *Cynomops planirostris*, 76%), (4) the western mastiff bat (*Eumops perotis*, 99%), and (5) the two *Miniopterus* species (*M. schreibersii* + *M. minor*, 96%). *T. tricolor* represents

the only lineage in the mammal phylogeny that traces through two independent gains (one in the ancestor of all bats, followed by loss in the ancestor of the 42-species clade that includes *M. aurita*, followed by a second independent gain in *T. tricolor*).

The common ancestor of all carnivores gained a baculum (99%), followed by two independent losses: one in the ancestor of aardwolf and two hyaena species (*Proteles cristata* + *Hyaena hyaena* + *Crocuta crocuta*, 100%) and one in the bearcat (*Arctictis binturong*, 100%) (Fig. 1G). It is interesting that the spotted hyaena lost the baculum, as this species has famously high levels of circulating androgens, even among females (Glickman et al. 1987).

We re-ran the stochastic mapping procedure after excluding the 35 species for which baculum presence/absence was inferred from a congeneric species. Our results changed very little, and we inferred a minimum of 8 independent gains and 10 independent losses. The reason we have one fewer gain species is because *T. syrichta* was one of the 35 species removed in this followup analysis, which leads to inference of a single, high confidence gain in the primate ancestor, supported by 67% of iterations. As comparison, we also ran a strict parsimony reconstruction of ancestral states, which revealed a minimum of 5 independent gains and 10 independent losses.

Boostrapping

The three versions of bootstrapping—uniform, proportion, and cophenetic—supported our general conclusions that the baculum has been gained and lost multiple times during mammalian evolution (Fig. 2). We, therefore, pooled the bootstrapping results. After subsampling 50%, 60%, 70%, 80%, and 90% of the species in our dataset, we observed a median (2.5–97.5% quantile) of 6 (1–10), 6 (2–11), 7 (3–11), 8 (5–11), and 9 (6–11) high confidence gains, respectively, and 7 (2–13), 8 (4–13), 8 (5–12), and 9 (6–11), 10 (7–10) high confidence losses, respectively. These numbers are close to those inferred from the whole dataset, where the baculum was gained a minimum of 9 times and lost a minimum of 10 times. In sum, the inference that the baculum has been gained and lost multiple times during mammalian evolution is robust to our exact sampling, and to unknown biases in taxonomic or phylogenetic studies.

Groups with a baculum diversify rapidly

Groups with a baculum diversify more rapidly than groups without (LRT = 58.7, df = 1, $P < 10^{-13}$). The

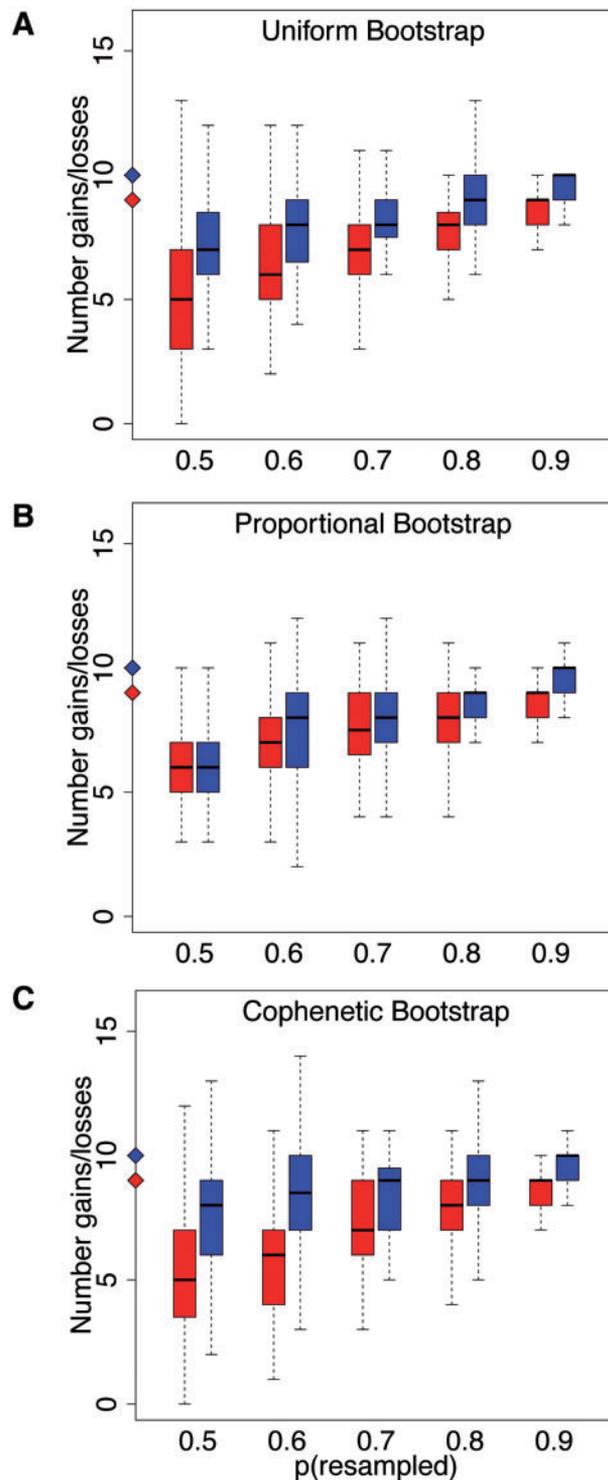


Fig. 2 Three different bootstrapping techniques were applied (see “Materials and methods” section). Following the color scheme in Fig. 1, red indicates baculum gain and blue baculum loss. Boxplots show the results of 100 iterations at each level of resampling. Red and blue diamonds indicate the 9 high confidence gains and 10 high confidence losses observed in the full dataset. (A) Uniform bootstrapping, (B) proportional bootstrapping, and (C) cophenetic bootstrapping. (This figure is available in black and white in print and in color at *Integrative And Comparative Biology* online).

full model estimated a rate of diversification in groups with a baculum that was more than three times higher than those without (0.071 vs. 0.022 new species per million years). However, there are two important caveats. First, the baculum might be correlated to the overall number of known species not because those groups diversify more rapidly, but simply because species with a baculum are easier for taxonomists to describe. One way to test this caveat is to score the number of species descriptions that rely on baculum morphology. Second, these methods assume that the phylogeny represents a random sample of all known extant species, which is almost certainly not the case. For example, we only included 3 of the roughly 100 species of cetaceans because although it is generally believed that cetaceans do not have a baculum, it was only specifically reported in three species (Supplementary Table 1). Adding 100 cetacean species without a baculum to our analysis would obviously increase the estimated rate of diversification among groups without a baculum. Therefore, we cautiously suggest that groups with a baculum might diversify more rapidly, but future expansion of our datasets are required to understand this pattern.

Discussion

The baculum is an extremely diverse morphological feature and there have been many attempts to uncover correlates between aspects of baculum morphology and mating ecology, with a roughly equal number of positive and negative results (Table 1). Due to publication bias, there are probably relatively more negative results that remain unknown, suggesting baculum characteristics are not strongly or consistently correlated with aspects of organismal biology. The studies highlighted in Table 1 include bats, rodents, carnivores, and primates. Our study shows that these four groups evolved their bacula independently, potentially reconciling an inconsistent literature. More specifically comparisons across groups assume that the structure being studied is homologous, inherited via common descent with modification, but our study clearly demonstrates this assumption is violated in studies of the baculum. Instead the baculum appears to have evolved under many different ecological contexts.

Multiple derivations

Specific details of our conclusions will probably be amended as more species are examined for the presence/absence of bacula and phylogenetic hypotheses expand. The baculum can be easily overlooked—for

example, the tiny baculum of the American pika (*O. princeps*) was only recently characterized through scanning electron microscopy, mass spectrometry, and cross-sectional histology (Weimann et al. 2014). However, given the phylogenetic distribution of bacula (Fig. 1), it is difficult to imagine that including more species in the future would overturn our main conclusion that the baculum evolved multiple times.

In the present study “baculum” refers to a bone in the penis, but this necessary simplification hides important heterogeneity. Bacula differ in their location in the penis and the distribution and type of ossification. Some bacula are expansive (Sharir et al. 2011), covering more than 75% of total penis length (Sinha 1976), while others are not (Hooper 1960; Rodriguez et al. 2011). Even the placement varies; while most bacula are dorsal to the urethra, and attach proximally to the corpora cavernosa (Rodriguez et al. 2011; Evans and de Lahunta 2013), the giant panda (*Ailuropoda melanoleuca*) baculum lies ventral to the urethra and does not attach to the distal aspect of the corpus cavernosum (Davis 1964).

The distribution of woven or lamellar bone in the baculum also varies across species. Lamellar bone has a more organized structure than woven bone, which enables it to be mechanically stronger (Bonewald et al. 2009). The mid-shaft of the rat baculum is mostly composed of dense lamellar bone, and shows signs of active bone remodeling, suggesting a role in load bearing (Kelly 2000). The shaft of the baculum in some bats is composed of lamellar bone surrounded by woven bone (Herdina et al. 2015b). In addition to the distribution of bone, the type of ossification in the baculum varies. Intramembranous, or direct, ossification occurs when undifferentiated mesenchyme is ossified, while endochondral ossification requires a cartilage intermediate (De Crombrugge and Akiyama 2009). Both types of ossification have been observed in distinct regions of the rat baculum during development (Murakami and Mizuno 1984), while only endochondral ossification is observed in other species (Smirnov and Tsytsulina 2003; Evans and de Lahunta 2013). Even patterns of ossification vary, ranging from simultaneous ossification arising from two distinct zones (Evans and de Lahunta 2013), in two zones at separate developmental stages (Murakami and Mizuno 1984; Yoon et al. 1990) or more numerous ossification centers (Callery 1951). Developmental timing of ossification also varies; the mouse baculum is barely visible in neonates (Glucksmann et al. 1976), while some bat

bacula develop by late embryonic stages (Smirnov and Tsytsulina 2003).

Taken together, these studies reveal heterogeneity in baculum development which would support the notion that bacula are not homologous structures. Unfortunately, detailed developmental data are lacking for most species.

Sexual selection

One model that is often invoked to explain the diversity of bacula is one of sexual conflict, whereby the baculum is a male “offensive” trait that continuously evolves to counteract female “defenses,” which could lead to recurrent adaptive evolution of both male and female traits. The morphological diversity of the baculum may in fact fit such a model, but it does not seem like the presence/absence of the baculum itself does. Instead, a gain or loss (Fig. 1) is often followed by long periods of evolutionary time without another transition. For example, the astonishing morphological diversity found in rodent bacula (Burt 1960) appears to have arisen against a backdrop of selective constraint maintaining presence of the bone, as no rodents have lost it.

Developmental biology

Our study brings up several important questions. First, do independent derivations of bacula proceed via switching on/off of conserved genetic pathways, or through the recruitment of novel molecular pathways? In sticklebacks, loss of pelvic girdles occurred via multiple independent mutations that affect expression of *Pitx1* (Bell 1987; Shapiro et al. 2004; Chan et al. 2010). Many genes involved in growth and patterning are shared between limbs and genital tubercles (Kondo et al. 1997; Cobb and Duboule 2005; Infante et al. 2015), and it is possible that some of these genes also affect baculum development.

There is evidence that species which have lost a baculum retain the developmental pathways to develop one. Hershkovitz (1993) identified vestiges of embryonic bacula in adult *Cacajao* (Primate, New World Monkey) specimens, the adults of which do not have bacula. Thus, bacula may be similar to other cases of arrested development followed by degeneration, including hind-limb regression in cetaceans (Thewissen et al. 2006), phallus regression in cloacal birds (Herrera et al. 2013), and the loss of teeth in birds (Harris et al. 2006). These examples show how loss of a trait as an adult is sometimes accompanied by early embryonic development of the trait, suggesting that conserved genetic pathways may

be poised for subsequent rederivation. Clearly, more studies are needed to elucidate the genetic basis of baculum variation.

Functional biology

Even a basic understanding of the function of the baculum will never be complete without knowledge of its precise interactions with the female during copulation. This is perhaps the largest obstacle to testing hypotheses of baculum function and evolution, as it requires observations of internal anatomy in naturally behaving, copulating animals. All bacula are thought to reside in the glans penis, which enters the female's reproductive tract during copulation (Hooper 1960). Male genital morphology has been shown to evolve in response to complexity of the female reproductive tract (Brennan et al. 2007, 2010; Higginson et al. 2012), with dramatic effects on paternity (Arnqvist and Danielsson 1999; House and Simmons 2003; Stockley et al. 2013; Dougherty et al. 2015). Herdina (2015a) showed that artificial inflation of the corpora cavernosa greatly altered the relative orientation of the bones to variable degrees in all three bat species tested, demonstrating that an understanding of the baculum must also take into account the effect of surrounding tissue and state of the penis. The number of erectile tissues and the degree to which they contribute to erections also varies in species with bacula (Christensen 1954; Davis 1964; Rodriguez et al. 2011), potentially adding even more diversity to baculum function.

Many, but not all, bacula have cartilaginous extensions on their most distal tip, some of which extend past the most distal aspect of the penis and also show tremendous morphological diversity (Hooper 1960; Rodriguez et al. 2011). Such cartilaginous structures may interact with the female reproductive tract (Meczynski 1974), or alter orientation of the penis to align properly during copulation (Evans and de Lahunta 2013). While these hypotheses remain untested, the variability in distal cartilage adds another layer of complexity to understanding the functional role of the baculum.

Conclusion

Most mammals have bacula, but we still understand very little about the function, development, and origin of this bone. The lack of consistent correlations to aspects of organismal biology leaves many questions unanswered. In the current study, we demonstrate that the baculum can no longer be considered a homologous structure in the traditional sense. Rather, multiple gains and losses of the bone suggest

species-specific responses to species-specific challenges. To reveal these biological challenges, future studies should focus on developmental and functional dissection of this remarkable structure.

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Supplementary Data

Supplementary Data available at *ICB* online.

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