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ORIGINAL PAPER

Genital morphology linked to social status in the bank vole (*Myodes glareolus*)

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Abstract Since genital morphology can influence the outcome of post-copulatory sexual selection, differences in the genitalia of dominant and subordinate males could be a factor contributing to the fertilisation advantage of dominant males under sperm competition. Here we investigate for the first time if penile morphology differs according to male social status in a promiscuous mammal, the bank vole (*Myodes glareolus*). In this species, dominant males typically achieve higher reproductive success than subordinates in post-copulatory sexual selection, and male genital morphology is complex, including both a baculum (os penis) and penile spines. Our results show that despite no difference in body size associated with male social status, baculum width is significantly larger in dominant male bank voles than in subordinates. We also found evidence of positive allometry and a relatively high coefficient of phenotypic variation in the baculum width of male bank voles, consistent with an influence of sexual selection. By contrast, baculum length and three measures of penile spinosity did not differ according to male social status or show evidence of positive allometry. We conclude that dominant male bank voles may benefit from an enlarged baculum under sperm competition and/or cryptic female choice and that differences in penile morphology according to male social status might be important but as yet largely unexplored source of variation in male reproductive success.

Keywords Allometry · Baculum · Genitalia · Penile spines · Sexual selection · Social dominance · Sperm competition

Introduction

Understanding the causes of variation in male reproductive success is a key goal of sexual selection research. Following Darwin's (1871) original insights, much attention has focused on the role of conspicuous sexually selected traits in influencing male mating success (Andersson 1994; Clutton-Brock 2007) under inter- or intra-sexual competition (e.g. Loyau et al. 2007; Husak et al. 2009). More recently, research has emphasised the role of less conspicuous male traits in increasing reproductive success under post-copulatory sexual selection. In particular, growing evidence suggests a role for male genitalia in promoting fertilisation success under both sperm competition (when sperm from two or more males compete for the fertilisation of a given set of ova; Parker 1970) or cryptic female choice (when females discriminate between sperm from different males; Thornhill 1983).

If genitalia influence male success in post-copulatory sexual selection, genital morphology might vary with male social status since dominant males often achieve higher fertilisation success under competitive conditions (Bretman

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et al. 2006; Klemme et al. 2006). Higher fertilisation success of dominant males may result from an advantage in sperm competition or cryptic female choice, which could be explained by differences in genital morphology linked to male social status, in addition to differences in frequency or timing of copulation (e.g. Nakamura 1998; Raveh et al. 2010) or differential ejaculate allocation (e.g. Cornwallis and Birkhead 2007; Thomas and Simmons 2009). However, despite growing evidence that genitalia have an important role in post-copulatory competition (Eberhard 1985, 1996; Hosken and Stockley 2004; Simmons et al. 2009), relationships of genital morphology and male social status have not yet been investigated.

The genital morphology of male mammals is diverse and includes variation in the presence and shape of the baculum (os penis) and keratinized spines on the surface of the penis (Eberhard 1985; Stockley 2002; Ramm 2007; Miller 2010). Bacula and spines are found in bats, carnivores, primates and rodents (Patterson and Thaeler 1982; Ryan 1991) and bacula are sometimes also present in eulipotyphlans (Burt 1960). Several functions associated with a role in post-copulatory competition have been proposed for these genital structures (Harcourt and Gardiner 1994; Stockley 2002; Ramm 2007; Dunham and Rudolf 2009). For example, a relatively large baculum could facilitate the placement of copulatory plugs in an optimal position (Toner and Adler 1986), deposit sperm close to the site of fertilisation (Ramm 2007) or facilitate intromissions and protect the urethra from compression during prolonged intromissions (Dixson 1987; 1995; Dixson et al. 2004). Several hypotheses also relate potential functions of the baculum and penile spines to stimulation of the female during copulation. For example, stimulation from penile spines might function to promote ovulation (Milligan 1979) or reduce the risk that females will remate (Stockley 2002).

In the present study, we investigated relationships of baculum morphology and penile spines with social status in the bank vole *Myodes glareolus* to determine whether penile morphology might be related to the greater success of dominant males of this species in post-copulatory competition (Klemme et al. 2006). Evidence for high levels of sperm competition in bank voles comes from both field and laboratory studies. The number of multiply sired litters has been estimated at 35.5% in wild populations (Ratkiewicz and Borkowska 2000) and females typically choose to mate with more than one male under laboratory conditions (Ratkiewicz and Borkowska 2000), regardless of male social status (Klemme et al. 2006). As in all rodents, the penis of male bank voles contains a baculum (Didier 1954), and the penis also has spines near its base (Milligan 1979). Moreover, there is ample opportunity for male genital structures to influence outcomes of post-copulatory sexual selection during copulatory contact since mating in this species typically consists of four to five ejaculatory series with multiple intromissions in each series (Borkowska 2010). We predicted that dominant male bank voles would have a larger baculum or more (or longer) penile spines than subordinates, which could potentially explain the higher reproductive success of dominant males. Sexually selected traits often are predicted to show positive allometry (Petrie 1992; Kodric-Brown et al. 2006; but see Bonduriansky 2007) and high variation (Petrie 1992; Pomiankowski and Møller 1995; Kodric-Brown et al. 2006), so we also tested for these patterns in bacular size and penile spines.

Materials and methods

Subjects

Subjects were first- and second-generation descendants of wild-caught bank voles trapped in Cheshire (UK). Breeding was managed to maximise genetic diversity in the captive population. After weaning, animals were housed with same-sex siblings in MB1 cages (45×28×13 cm, North Kent Plastic Cages Ltd, UK) containing substrate (Corn Cob Absorb 10/14 substrate) and paper-wool nest material. All male subjects thus had equivalent social experience at the start of the study and none had previously mated. Food and water were provided ad libitum (LabDiet 5002 Certified Rodent Diet, Purina Mills, St Louis, MO, USA). Animals were maintained on a reversed photoperiod (light, 16 h; dark, 8 h; lights on at 1700 h) and at a temperature of 21±1°C. This research adhered to the Association for the Study of Animal Behaviour/Animal Behaviour Society guidelines for the Use of Animals in Research (http://asab.nottingham.ac.uk/ethics/guidelines.php), the legal requirements of the country in which the work was carried out and all institutional guidelines. No specific licences were required for this work.

Assessing dominance relationships

We assessed dominance following Horne and Ylönen (1996) and Klemme et al. (2006) by pairing non-sibling males in MB1 cages divided in two by a mesh barrier, with one male of each pair housed in each half of the cage (i.e. in a 45×14×13-cm area) over a period of 3 to 5 weeks. The mesh barrier allowed continuous olfactory, visual and auditory contact between males while they were housed together. To assess scent marking behaviour and assign social status to subjects, both males from each pair were transferred to clean Benchkote-lined MB1 cages (again divided in two by a mesh barrier) and left for 30 min during the dark phase. Scent marks were scanned using a Bio-rad Fluor-S™ MultiImager (QuantityOne software: 12-s exposure, 530DF60 Filter, UV light source Epi illumination, high-resolution mode). The social status of males was assessed using the criteria of Rozenfeld and Rasmont (1991; see also Horne and Ylönen 1996). Specifically, thin streaks of urine deposited throughout the arena are characteristic of dominant males. By contrast, subordinates either deposit large pools of urine, especially in corners of the arena (Rozenfeld et al. 1987; Rozenfeld and Rasmont 1991; Klemme et al. 2006), or they deposit no urine marks (Rozenfeld and Rasmont 1991). Nest material from an unrelated female (approximately 13 g) was added once to each compartment of the divided cages, 1 day prior to the first collection of scent marks from each male pair, to increase male competitiveness (Kruczek 1997). Male roles as dominant and subordinate were assigned when a clear and consistent difference in the pattern of scent marks within a pair was apparent for three successive scent-marking assays (each conducted at least 24 h apart). Then, males were kept paired until the end of the experiment. If no clear dominant–subordinate pattern could be identified within a pair, males were rehoused in their original cages or paired with a new unrelated male. Measurements of paired preputial gland mass (measured post-mortem, see the following text discussion) subsequently confirmed our classification of male social status based on scent marking behaviour. Males classed as dominant had significantly heavier preputial glands (dominants: $\overline{X}$= 45.52±4.73 mg; subordinates: $\overline{X}$ = 22.51±2.21 mg; t_{46} =; $P<0.001$), consistent with evidence that high social rank is associated with large preputial glands in this species (e.g. Christiansen et al. 1978; Kruczek 1997). Dominant ($n=25$) and subordinate ($n=23$) males did not differ significantly in age (dominants: $\overline{X}$ = 250.04±13.71 days; subordinates: $\overline{X}$ = 233.96±17.34 days; $t_{46}=0.73$; $P=0.47$). A difference in sample size between dominant and subordinate males occurs in our analyses because two subordinate subjects died before completion of the study.

Preparation and measurements of bacula and metatarsal bones

As in many rodents, the bank vole baculum is complex (Fig. 1), with a stalk that is broader at its proximal end than at its distal end (Artimo 1964). This structure is then articulated at its distal extremity with three digital processes through a synovial joint (Artimo 1964; Arata et al. 1965). We measured the baculum and third metatarsal bone of the right hind foot. Metatarsal bones were used as a control trait, unlikely to be influenced by sexual selection (Ramm et al. 2010), and were measured from a different subset of males of similar age from the same population. Subjects were killed with an overdose of halothane. Measures of body mass (to ± 0.1 g) and male body length (to ± 0.1 mm) were taken post-mortem using an electronic balance and digital callipers. The penis of each male was removed and frozen prior to further analysis. Penises were defrosted at room temperature and most tissue was removed manually with forceps at ×20 magnification. Following Friley (1947) and Tasikas et al. (2009), further dissections were combined with soaking for 1–2 h in 1 ml of KOH at a concentration of 0.05 gml^{-1}. Cleaned bacula were stored in 1 ml of 70% ethanol solution. An identical procedure was used for removal and cleaning of metatarsal bones.

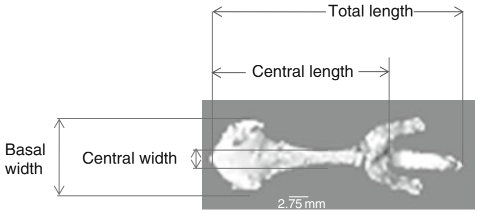

Fig. 1 Ventral view of the bank vole baculum. To measure the baculum, we followed the same method as that of Tasikas et al. (2009) for four traits: total length, central length, central width, and basal width. The central length variable measures the stalk length of the baculum from the proximal to the distal extremity

The bank vole baculum is morphologically similar to that of the common muskrat (*Ondatra zibethicus*), so we followed the measurement procedure of Tasikas et al. (2009). Four measurements (to ± 0.01 mm) were taken (Fig. 1): total length, central length, central width and basal width. We also measured the length and width of the metatarsal bone to compare our results with a non-genital bone (Ramm et al. 2010) (both measure to ± 0.01 mm). Measurements were taken from digitised images made with a flatbed scanner (CanoScanLiDE 30, Canon Inc.) at a resolution of 1,200 dpi (Ramm et al. 2010). Scans were imported into ImageJ software (version 1.38x, http://rsbweb.nih.gov/ij/), inverted and converted to 32 bit. To facilitate measurement, we rotated the pictures to align the bacular shaft on a vertical axis. Measurements were not taken from conspicuously damaged bacula, which explains variation in sample size. Two researchers (JFL and NJ) performed the measurements independently and their results were highly reliable (e.g. for baculum total length: intra-class correlation coefficient, ICC=0.83; $F_{1,\ 19}=12.49$; $P<0.001$).

Preparation and measurement of penile spines

Samples from 20 males (ten dominants and ten subordinates) were first placed in frozen fixative (2.5% glutaraldehyde, 4% formaldehyde in 100 mM sodium phosphate buffer) to thaw at room temperature. After a night of fixing at room temperature on a rotator, samples were washed twice (40 min each time) with a solution of 100 mM sodium phosphate buffer. Next, samples were submitted to a series of dehydration (45 min each) with ethanol of increasing concentration (25%, 50%, 70%, 90% and 2×100%) and were treated with hexamethyldisilazane (HMDS) for 1 h. After this, most of the HMDS was removed and the remainder was then allowed to evaporate off in a desiccator overnight. Penises were mounted using silver-DAG (conductive silver paste) on standard aluminium stubs for scanning electron microscopy and sputter-coated with gold/palladium. Finally, specimens were viewed using a scanning electron microscope JSM 6490LV (JEOL, Tokyo, Japan) running at 5 kV, and images were taken at low magnification (Fig. 2). To determine the different information needed, images were taken at ×30 magnification to see the overall specimen, at ×60 to show the whole band of spines and at ×200 to determine the density of spines.

Spines were investigated using ImageJ software. The ×30 pictures were used to estimate the percentage of the penis surface covered by spines (hereafter called spine coverage) (Fig. 2). To determine spine length, 15 spines on the ×200 pictures were measured and the mean of these values was calculated. Size was measured from the tip to the middle of the base of the spines (to ± 1 μm) (Fig. 2c). Finally, to determine spine density, all spines were counted (using the Cell Counter Plugin for ImageJ) on the ×200 pictures and divided by the surface area they covered.

Statistical analysis

Allometry was analysed with ordinary least squares (OLS) regression on log-transformed data. OLS regression tests if the slope of the regression line deviates significantly from zero (H_0: $\beta=0$). Transformed data for each trait were entered in the models as dependent variables with transformed data for body length as the independent variable. However, since the OLS regression assumes no measurement error for the *x*-axis (Sokal and Rholf 1995), we used reduced major axis (RMA) regression (model II) to estimate unbiased slopes of regression lines (Lüpold et al. 2004; Tasikas et al. 2009; Ramm et al. 2010). *T*-tests assessed if the estimated slopes differed from isometry (H_0: $\beta=1$) (Eberhard et al. 1998; Lüpold et al. 2004).

To compare variation across traits, the coefficient of variation (CV) was calculated as (s.d./mean)×100. CV′ was also calculated as $CV^*(1-r^2)^{1/2}$, with r being the Pearson correlation coefficient between the studied trait and body length (Eberhard et al. 1998). CV′ is the coefficient of variation of the investigated trait if body size is held constant (Eberhard et al. 1998). As emphasised by Eberhard et al. (1998, 2009), it is important to be cautious in the interpretation of the coefficient of variation and values should normally be interpreted in comparison to those for other traits.

Since it has been shown that the baculum can grow after sexual maturation (Arata et al. 1965), we took into account the effect of male age on baculum measurements. Hence, we ran multiple regression analyses including each bacular measurement as a dependent variable and male body size, male age and male body size × age as independent variables. These and other statistical tests were conducted

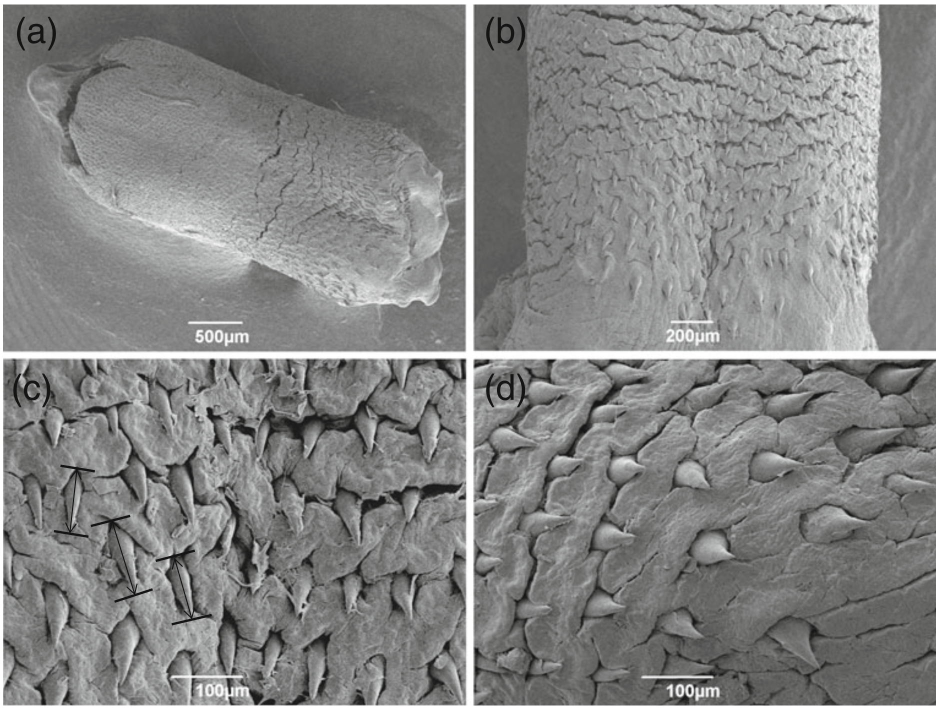

Fig. 2 Scanning electron microscope images of the adult bank vole penis. In **a**, the whole penis can be seen from its distal end (on the *left*) to its proximal end (on the *right*), whereas in **b** only the surface of the penis covered by spines is shown, located towards its proximal end. In **c** and **d**, the morphology of the penile spines is more clearly visible (*arrows* in **c** show length measurements taken on individual spines, from the tip to the middle of the base of this structure). The magnification of **a** is ×30, of **b** ×60 and of **c** and **d** ×200

on log-transformed data using SPSS 18.0. Data are presented as means ± s.e.m (except for coefficients of variation which are presented as means ± s.d.). All tests are two-tailed and the significance level is $\alpha=0.05$.

Results

Genital morphology, body mass and male social status

Dominant and subordinate males did not differ in body mass (dominants: $\overline{X}=26.5\pm0.9$ g; subordinates: $\overline{X}=24.8\pm0.8$ g; $t_{46}=1.27$; $P=0.21$) or body length (dominants: $\overline{X}=98.6\pm1.1$ mm; subordinates: $\overline{X}=96.8\pm1.1$ g; $t_{46}=1.20$; $P=0.24$), and male age had no effect on any of the bacular variables measured (Table 1). A comparison of each genital trait measured for dominant and subordinate male bank voles is presented in Table 2. Consistent with our predictions, baculum morphology differed significantly according to male social status, with the basal width of the baculum significantly larger in dominant males compared to subordinates (dominants: $\overline{X}=1.34\pm0.05$ mm; subordinates: $\overline{X}=1.18\pm0.04$ mm; $t_{27}=2.40$, $P=0.02$). Although the difference is marginally non-significant, dominant males tend to have also a larger central baculum width compared to subordinates (dominants: $\overline{X}=0.34\pm0.01$ mm; subordinates: $\overline{X}=0.30\pm0.01$ mm; $t=1.90$, $P=0.07$).

Including body length (or body mass) as a covariate in general linear models to explore the effects of social status on baculum morphology gives similar results to those presented above without control for body size. For example, with body length as a covariate, social status has a significant effect on baculum basal width ($F_{1,\ 27}=5.56$, $P=0.03$) but not on baculum central length ($F_{1,\ 24}=0.01$, $P=0.90$). However, dominant and subordinate males did not differ in total or central length of the baculum (Table 2). Similarly, we found no evidence that variation in penile spines is linked to male social status since neither the size, density or coverage of penile spines was significantly different between dominant and subordinate males (Table 2).

Table 1 Influence of male age and body size on bacular measurements in the bank vole. All results are qualitatively unchanged when the interaction body size × age is removed from the models

Baculum measurement	Estimate ± S.E.M.	t	P
Total length ($n=21$)			
Body size	0.001 ± 0.001	1.15	0.26
Age	$1.24\times10^{-5}\pm0.1\times10^{-5}$	0.13	0.90
Body size×age	$-2.20\times10^{-5}\pm0.1\times10^{-5}$	−1.05	0.31
Central length ($n=26$)			
Body size	0.003 ± 0.001	2.46	0.02
Age	$-8.39\times10^{-5}\pm0.1\times10^{-5}$	−0.85	0.40
Body size×age	$-1.30\times10^{-5}\pm0.1\times10^{-5}$	−0.55	0.59
Central width ($n=33$)			
Body size	0.004 ± 0.002	1.50	0.14
Age	$< 1\times10^{-5}\pm0.1\times10^{-5}$	0.72	0.47
Body size×age	$-2.96\times10^{-5}\pm0.1\times10^{-5}$	−0.65	0.52
Basal width ($n=29$)			
Body size	0.006 ± 0.002	2.71	0.01
Age	$8.12\times10^{-6}\pm0.1\times10^{-6}$	0.05	0.96
Body size×age	$-5.19\times10^{-5}\pm0.1\times10^{-5}$	−1.33	0.2

Allometry and variation

Consistent with certain models of sexual selection, slopes for baculum basal width were significantly greater than 0 with ordinary least squares regression and greater than 1 ($\beta_{\mathrm{RMA}}=3.44$) with the reduced major axis regression. Slopes for baculum central length were significantly greater than 0 (OLS regression) but not greater than 1 (RMA regression), and slopes for baculum central width and total length were not significantly greater than zero with OLS regressions (Table 3). Hence, the allometric slope for baculum basal width is steeper than for the other baculum dimensions (total length, central length and width) and this trait shows a pattern of positive allometry. We found no evidence of positive allometry for penile spine length and none of the allometric slopes for metatarsal traits (length and width) was significantly greater than 0 (Table 3).

As predicted under certain conditions for structures influenced by sexual selection, both width dimensions of the baculum had a relatively high coefficient of phenotypic variation (CV′) (>13%) compared to control traits such as metatarsal length (2.3%) or width (10.6%) (Table 3). Among all genital structures investigated, penile spine length had the highest coefficient of phenotypic variation (20.0%), whereas for baculum central and total length the coefficient of phenotypic variation was less than 10 % (6.2% and 7.6%, respectively) (Table 3).

Discussion

Our study provides indirect evidence that baculum width may influence male reproductive success in bank voles. Dominant males have higher reproductive success than subordinates under post-copulatory sexual selection (Klemme et al. 2006; Kruczek and Zatorska 2008), and here we found that dominant males have a larger baculum basal width than subordinates. Given the likely diverse functions of the baculum in post-copulatory sexual selection (Eberhard 1985; Harcourt and Gardiner 1994; Hosken and Stockley 2004; Ramm 2007; Dunham and Rudolf 2009), it would therefore be of interest to determine if

Table 2 Comparison of age, body mass, body size and genital morphology according to social status of male bank voles. Data are shown as mean ± s.e.m. (*n*)

Trait	Dominant	Subordinate	*t*	*P*
Age (days)	250.04±13.71 (25)	233.96±17.34 (23)	0.73	0.47
Body mass (g)	26.5±0.9 (25)	24.8±0.8 (23)	1.27	0.21
Body length (mm)	98.6±1.1 (25)	96.8±1.1 (23)	1.20	0.24
Bacular measurements (mm)				
Total length (mm)	4.04±0.06 (10)	3.92±0.09 (11)	1.17	0.25
Central length (mm)	2.71±0.07 (14)	2.72±0.06 (12)	−0.08	0.94
Central width (mm)	0.34±0.01 (16)	0.30±0.01 (17)	1.90	0.07
Basal width (mm)	1.34±0.05 (14)	1.18±0.04 (15)	2.40	0.02
Spine measurements				
Length (μm)	70.59±3.67 (10)	72.35±5.43 (10)	−0.27	0.79
Coverage (%)	32.01±1.64 (10)	29.93±3.14 (10)	0.59	0.56
Density (spines/μm²)	103.32±5.57 (10)	95.68±8.31 (10)	0.76	0.45

baculum width influences male fertilisation success under competitive conditions. For example, a wider baculum might provide increased stimulation to the female during copulation, resulting in increased fertilisation success. Moreover, female bank voles are induced ovulators, meaning that mechanical stimulation provided by males during copulation causes ovulation (Clarke et al. 1970). A wider baculum might therefore also lead to an increased ovulation rate in response to stimulation from dominant males.

The larger bacula of dominant males in this study was not explained by body size per se—that is, dominant males did not have larger bacula because they were larger overall compared to subordinate males. However, dominant male bank voles typically invest more than subordinates in other traits linked to success in mating and sperm competition, such as testis size or sperm quality (Kruczek and Zatorska 2008; Kruczek and Styrna 2009; Lemaître et al. unpublished data), perhaps because they are better able to support increased investment in costly reproductive traits. Hence, in relation to the present study, it is possible that investment in the baculum could also be condition dependent or costly to some extent, although evidence in

Table 3 Phenotypic variation and allometry of genital and non-genital morphology in bank voles. Statistics are given for ordinary least square and, where appropriate, reduced major axis regressions

Trait (mm)	Individuals	*n*	Phenotypic variation					OLS (H_0: slope=0)			RMA (H_0: slope=1)		
			Mean	s.d.	CV (%)	CV′ (%)	*r*	Slope	s.e.	*t*	Slope	s.e.	*t*
Baculum total length	All	21	3.98	0.25	6.33	6.16	0.23	0.26	0.25	1.03	–	–	–
	Dominants	10	4.05	0.2	4.94	4.53	0.4	0.35	0.28	1.22	–	–	–
	Subordinates	11	3.92	0.29	7.4	7.4	0.03	0.04	0.45	0.08	–	–	–
Baculum central length	All	26	2.71	0.23	8.49	7.62	0.44	0.64	0.27	2.37*	1.48	0.27	1.75
	Dominants	14	2.71	0.25	9.22	8.09	0.48	0.73	0.39	1.87	–	–	–
	Subordinates	12	2.72	0.23	8.46	7.68	0.42	0.64	0.43	1.46	–	–	–
Baculum central width	All	33	0.32	0.05	16.98	16.4	0.26	0.76	0.50	1.52	–	–	–
	Dominants	16	0.34	0.05	14.71	13.99	0.31	0.80	0.65	1.24	–	–	–
	Subordinates	17	0.3	0.05	16.67	16.43	0.17	0.49	0.74	0.65	–	–	–
Baculum basal width	All	29	1.26	0.19	14.92	13.54	0.42	1.05	0.44	2.38*	2.52	0.44	3.44**
	Dominants	14	1.34	0.19	14.18	11.01	0.63	1.47	0.53	2.78*	2.36	0.53	2.56*
	Subordinates	15	1.18	0.15	12.71	12.65	0.1	0.22	0.64	0.35	–	–	–
Spine length	All	20	0.07	0.01	20	19.96	0.06	−0.35	1.39	−0.25	–	–	–
	Dominants	10	0.07	0.01	14.29	12.76	0.45	−1.80	1.26	−1.43	–	–	–
	Subordinates	10	0.07	0.02	28.57	28.05	0.19	1.43	2.64	0.54	–	–	–
Metatarsal length		27	7.24	0.17	2.29	2.29	0.005	−0.003	0.11	−0.03	–	–	–
Metatarsal width		27	0.56	0.06	10.79	10.63	0.17	0.47	0.54	0.88	–	–	–

$^*P<0.05$; $^{**}P<0.01$

support of this idea is currently limited (Ramm et al. 2010). Also consistent with a potential influence of sexual selection on baculum width, we found evidence of both positive allometry and high variation in baculum basal width. Moreover, when comparing patterns of allometry for dominant and subordinate males, positive allometry of baculum basal width was evident only among dominant males. Hence, it appears that larger male bank voles may invest in a larger baculum only when they are dominant. Positive allometry is commonly reported for sexually selected traits such as ornaments and weapons (Green 1999; Kodric-Brown et al. 2006), and similar patterns have been used to infer evidence of sexual selection acting on genital structures, particularly in mammals (e.g. Kinahan et al. 2007; Tasikas et al. 2009). However, it is important to be cautious in the interpretation of allometric patterns since positive allometry is not necessarily a consequence of sexual selection nor does directional selection necessarily lead to the evolution of positive allometry (Bonduriansky 2007; Eberhard 2009).

Currently, it is difficult to generalise our findings to other species since this is to our knowledge the first time that variation in penile morphology has been found in relation to male social status. However, the width of the baculum (as opposed to its length) has previously been included in some allometry studies (e.g. Miller and Nagorsen 2008; Tasikas et al. 2009), and the pattern of positive allometry that we report here for baculum basal width in bank voles is similar to that found in some other rodents (e.g. *Bathyergus suillus*, Kinahan et al. 2007; *O. zibethicus*, Tasikas et al. 2009; *Mus musculus domesticus*, Ramm et al. 2010) and carnivores (e.g. *Martes caurina*, Miller and Nagorsen 2008). This suggests that the width of the baculum might play a more important role in mammalian post-copulatory competition than has previously been considered.

Baculum length (central and total) did not differ according to social status of male bank voles in our study, suggesting that this trait is unlikely to be a factor explaining the higher reproductive success of dominant males in post-copulatory competition (Klemme et al. 2006; Kruczek and Zatorska 2008). Moreover, variation in baculum length is relatively low and in the same range of values as traits assumed to be non-sexually selected (Pomiankowski and Møller 1995; House and Simmons 2003; but see Eberhard et al. 2009). These results contrast to some extent with the pattern of positive allometry and high coefficient of phenotypic variation exhibited by baculum length in several other mammals (e.g. Miller and Burton 2001; Kinahan et al. 2007; Tasikas et al. 2009; but see Ramm et al. 2010). However, the absence of positive allometry does not necessarily preclude sexual selection and there are ongoing discussions on the link between sexual selection on a trait and the pattern of allometry exhibited by that trait (reviews in Bonduriansky 2007; Eberhard 2009; Eberhard et al. 2009).

Similar to baculum length, the degree of penile spinosity of male bank voles did not differ in relation to social status, and we found no evidence of positive allometry in the size of penile spines. Although the function of mammalian penile spines is uncertain, it is often hypothesised that they have evolved under sperm competition or sexual conflict (Hosken and Stockley 2004; Dunham and Rudolf 2009). For example, penile spinosity is negatively associated with the duration of female receptivity in primates and may function to reduce sperm competition risk (Stockley 2002). Moreover, a high level of penile spinosity has been linked to an induced mode of ovulation in certain rodents (e.g. African mole rat species; Parag et al. 2006). Since bank voles are promiscuous and induced ovulators, penile spines might therefore function in either or both of these contexts, or in the removal of copulatory plugs (Stoddart 1979). Although we found no evidence of sexual selection on penile spines in the present study, a function in the context of mating competition cannot be ruled out. For example, if spines function to reduce the risk of female remating or promote ovulation by providing stimulation during copulation, it is possible that their stimulatory efficiency might be enhanced by a larger baculum (or penis) or by male copulatory behaviour rather than by an increase in spine length or density. Further studies are thus needed to investigate in more detail the function of these spines, their relationships with other genital structures and their role, if any, in sexual selection.

In conclusion, the results of our study demonstrate that the penile morphology of male bank voles differs predictably according to their social status. Dominant males have a larger (wider) baculum, which might confer an advantage in post-copulatory competition and might at least partly explain their higher reproductive success. Moreover, partly consistent with some other mammals, baculum basal width in bank voles shows positive allometry and a relatively high coefficient of phenotypic variation, which further suggests a potential influence of sexual selection on this trait (Pomiankowski and Møller 1995; Kodric-Brown et al. 2006; but see Bonduriansky 2007). Importantly, however, mechanistic information on the role of the baculum during copulation is still missing, and extreme interspecific diversity in baculum morphology remains largely unexplained (Burt 1960; Hooper and Hart 1962; Miller 2010). Future experiments should therefore investigate the role of baculum morphology in explaining variation in reproductive success according to male social status in bank voles and explore the extent to which these results can be generalised to other mammalian species.

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