

Bat genitalia: allometry, variation and good genes

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Male genitalia are typically highly variable across species, for which sexual selection is thought to be responsible. Sexually selected traits characteristically show positive allometry and high phenotypic variation, although genitalia seem to be typified by negative allometry due to stabilizing selection. Additionally, while sexual selection appears to be the primary force responsible for genital evolution, the precise mechanism is unclear, but good-genes selection could be involved. If so, male genital variation should correlate with some male quality measure(s). We investigated the allometry of male *Nyctalus noctula* genitalia and investigated associations between genital size and three phenotypic measures of male quality (body size, relative body mass, and fluctuating asymmetry (FA)). We found that the penis exhibited positive allometry and high phenotypic variation, and was positively associated with male body size and relative body mass, but not with FA. This pattern is more typical of sexually selected display traits, contrasting with general patterns of genital allometry. The baculum was negatively allometric and was not associated with any quality measure. Our results suggest that the *N. noctula* penis is under directional sexual selection and is a reliable indicator of male phenotypic quality. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, 83, 497–507.

ADDITIONAL KEYWORDS: baculum – Chiroptera – fluctuating asymmetry – museum specimens – penis – sexual selection.

INTRODUCTION

Male genitalia, including the mammalian baculum (os penis), are typically highly variable across species. This diversity implies rapid and divergent evolution, and although various mechanisms have been invoked to explain this variation, sexual selection is now generally accepted as being the force responsible (Eberhard, 1985; Arnqvist, 1998; Danielsson & Askenmo, 1999; House & Simmons, 2002; Hosken & Stockley, 2004). Sexually selected traits often show positive allometry (Petrie, 1988, 1992; Green, 1992; Simmons & Tomkins, 1996; Knell, Pomfret & Tomkins, 2004) and exhibit high degrees of phenotypic variation as a result of directional sexual selection (Pomiankowski & Møller, 1995). Genitalia are an exception to this general pattern and tend to be negatively allometric (e.g. Eberhard *et al.*, 1998; Tatsuta, Mizota & Akimoto,

2001; Bernstein & Bernstein, 2002; reviewed in Hosken & Stockley, 2004). The most likely explanation for this pattern is that unless there is size assortative mating, males are selected to fit average-sized females (Eberhard *et al.*, 1998). This results in stabilizing selection and hence negative genital allometry and low variability in genital size within species. However, most investigations of genital allometry have been conducted on insects (e.g. Eberhard, 1985), and, in contrast to the general pattern, two recent investigations of genitalia in vertebrates documented positive genital allometry (Kelly, Godin & Abdallah, 2000; Miller & Burton, 2001). This serves to highlight the problem of drawing broad conclusions on genital evolution when the taxonomic representation of investigations is not truly representative. Hence, further studies of genitalia are required to assess the generality of these apparent differences between vertebrates and insects.

It has also been argued that Fisherian processes are largely responsible for sexual selection on genitalia (Eberhard, 1993). However, other sexual selection mechanisms may also be involved (Simmons, 2001;

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Hosken & Stockley, 2004). For example, females could obtain good-genes benefits if genital size is condition-dependent and high-quality males have larger genitalia (Arnqvist, 1997; Eberhard *et al.*, 1998; Simmons, 2001). One proposed case of good-genes sexual selection acting on genitalia is that of the harp seal (Miller & Burton, 2001). Unlike the typical pattern of negative genital allometry, the seal baculum displays a strong positive allometric association with body size. Seals often mate aquatically (e.g. Wilmer *et al.*, 1999 and references therein), and while such matings are largely under female control, they apparently hinder visual assessment of male quality. Thus, it was suggested that the size of a male's penis provides females with information about the male's body size and quality, leading to the positive allometry between baculum and body size (Miller & Burton, 2001). Similar to seals, torpid female bats may not be able to choose mates actively when copulation occurs during hibernation. Therefore, it is possible that females cryptically (in the broadest sense) choose males based on genital size (i.e. after intromission; Eberhard, 1996). For example, males with a longer penis may be the only ones able to bypass the uropatagium (tail membrane) of hibernating females.

If male genitalia were subject to good-genes sexual selection, associations between genitalia size and indicators of male quality are expected. There are three frequently used phenotypic measures of quality in evolutionary biology (Blanckenhorn & Hosken, 2003): body size (Andersson, 1994; David *et al.*, 2000; McEligott *et al.*, 2001), size-corrected body mass (Jakob, Marshall & Uetz, 1996; Kotiaho, 1999; García-Berthou, 2001) and fluctuating asymmetry (FA: small random deviations from perfect symmetry in bilaterally paired morphological features; Van Valen, 1962; Palmer & Strobeck, 1986; Møller, 1990). Of these measures, FA is the most contentious because of contradictory results across studies (Hunt & Simmons, 1997; Clarke, 1998; Bjorksten, Fowler & Pomiankowski, 2000; Fuller & Houle, 2002; Tomkins & Simmons, 2003), and because FA tends to be trait-specific rather than being consistent organism-wide (e.g. Palmer, 1994; Bjorksten *et al.*, 2000; Hosken, Blanckenhorn & Ward, 2000). Nevertheless, associations between sexual selection and FA exist (Møller, 1992, 1993; Thornhill, 1992; Swaddle & Cuthill, 1994; Hunt *et al.*, 2004; see Hosken, 2001). Additionally, Palmer & Strobeck (1986) suggested that functionally important characters should show less FA than do less important traits. For example, the wings of flying animals should attain the highest possible level of symmetry because of the large effects of asymmetry on flight performance (Norberg & Rayner, 1987; Balmford, Jones & Thomas, 1993; Thomas, 1993; Gummer & Brigham, 1995; Swaddle, 1997).

We addressed a number of questions relating to the genital evolution of the noctule bat *Nyctalus noctula*. Firstly, we asked: how do *N. noctula* genitalia (the penis and baculum) scale with body size, and does genital scaling differ from that of non-genital traits? We then asked: are there associations between the size of the *N. noctula* penis and baculum and phenotypic measures of male quality (FA, size and size-corrected mass)? Finally, we asked: is FA trait-specific, and is it lower for traits likely to be functionally more important? *N. noctula* is a good model with which to investigate these questions for a number of reasons: it has a large baculum, females mate repeatedly with different males and store sperm during hibernation (Racey, 1979; Mayer, 1997; McCracken & Wilkinson, 2000), and copulation occurs during torpor (Gebhard, 1997). This reproductive system, together with delayed fertilization, is likely to lead to intense postcopulatory sexual selection, as reflected by the large testes (Hosken, 1997). Furthermore, symmetry is likely to be important, especially in the wings. Therefore, if FA indeed reflects quality, it should be obvious in this species. We employed body size and size-corrected body mass as additional phenotypic measures of male quality.

MATERIAL AND METHODS

We used 80 adult male and 31 adult female *N. noctula* that had been collected from the Swiss midlands between 1979 and 1995, and fixed and preserved in 70% ethanol at the Zoological Museum of the University of Zürich. Specimens were selected from two periods of the year, winter (November–February) and early to mid summer (May–August). Samples which had broken or missing parts or otherwise unmeasurable traits were excluded from measurement for the affected traits, so sample sizes vary for the different traits and analyses. Limb, cranial and male genital characters, body length and mass were used to estimate allometric or trait/quality relationships and/or levels of FA. Note that after adult size has been attained, the baculum of *N. noctule* no longer grows (Smirnov & Tsytsulina, 2003).

DISSECTIONS

After the penis length of all males had been measured to the nearest 0.15 mm, using the ocular scale of a dissection microscope ($\times 6.4$), the baculum was removed and stored in proteinase K (100 $\mu\text{g}/\text{mL}$) at 55 °C for c. 1 day. Subsequently, digital images of the cleaned baculum were taken for measurement. Regression comparisons (measure 1 on measure 2) of these images with some taken immediately after dissection, and thus before cleaning, indicated that this treat-

ment did not significantly affect bacular shape or size (d.f. = 14; $b = 0.94$; $r^2 = 0.89$; t ($H_0: b = 1$) = -0.72 ; $P > 0.3$).

The skull was removed, cleaned using forceps and bleach (13–14% vol. in water), degreased in a detergent–water solution, and bleached in hydrogen peroxide (1%) before being air-dried.

MEASUREMENTS

All measurements were taken by the same person (S.L.). Body length was measured to the nearest 0.5 mm using a piece of string held taut along the spine from the mouth to the base of the tail ($N = 80$). Body mass was measured on a digital balance to the nearest 0.01 g after 1 h of air drying ($N = 54$) (sample sizes varied here due to variable preservation/completeness of specimens). The reliability of the relative body masses of the preserved *N. noctula* was assessed by comparing them with data from 80 live *N. noctula* (J. Gebhard, unpubl. data) measured in the wild during the same two periods of the year (May–August and November–February). A general linear model (GLM) with forearm length and state (dead or live) as predictor variables and body mass as the dependent variable revealed that only forearm length had a significant effect ($F_{1,130} = 8.44$, $P = 0.004$), and neither state nor the interaction (state \times forearm) was statistically significant (all $F < 0.9$; all $P > 0.34$). Therefore, there was no indication that the body mass of our specimens significantly differed from that of live *N. noctula*. Note that body mass was measured prior to penis removal, which opens up the potential problem of part–whole correlations. Additionally, we could not go reweigh the penis with any degree of accuracy because of subsequent dissection to remove the baculum. However, we calculate that the penis would have to weigh more than 7% of the total body mass before our interpretation of the results presented here would be affected, and this is inconceivable. Based on the measurement of four adult *N. noctula* that were not included in this study, we found the intact penis to represent only 0.18% of the body mass. As a result we are confident that the results presented here are not biased by part–whole-correlation problems.

The length of the forearms, fingers (first phalanx of third digit) and tibiae was measured on both sides of the body to the nearest 0.01 mm using a set of digital hand calipers. Other traits (toe length, baculum and cranial traits) were measured from photographs using NIH Image 1.62 (US National Institute of Health). Toe length was measured from the base of the toe to the proximal end of the claw. Baculum length was measured from the distal tips to the proximal ends of the lobes. However, for FA analyses, only baculum lobe length was used, as this was the only part that had

clear left and right components. Four skull measures were taken: the lengths of the zygomatic foramen and mandible, the distance between the tips of the angular and coronoid processes (henceforth referred to as angular–coronoid), and the distance from the tip of the condylar process to the posterior end of the tooth row (referred to as condylar–molar). Lower jaws and skulls were digitized separately, and the mandibles were not separated at the frontal symphysis. The buccal side of each mandibular ramus was positioned flat onto a ruler blade, which laid across two identical small boxes, with the other ramus hanging down from the ruler. For images of the skulls, these were placed upside down in a small box filled with dark fine-grained sand. They were adjusted such that both jugal bones were parallel to and level with the rim of the box.

STATISTICS

To assess measurement error, all traits were measured twice or three times on either side of the body, with an interval of at least 2 days between repeated measurement sessions. The two or three values for each side were averaged for the analyses of FA after statistically separating asymmetry from measurement error. The measurement error was determined by estimating the proportion of within-individual variation relative to the total variation (Bailey & Byrnes, 1990; Sokal & Rohlf, 1995).

For the analyses of allometry (log–log regressions of trait size on body length), linear ordinary least squares (OLS) were employed to determine whether slopes significantly differed from zero (Tatsuta *et al.*, 2001). If these slopes were significant, reduced major axis regression (RMA) was used to test for significant deviations from isometry ($\beta = 1$). Reduced major axis regression is preferable to OLS if both the dependent and the independent variables are subject to measurement error (Sokal & Rohlf, 1995; Green, 1999), as was the case here. Furthermore, baculum and penis length were tested for associations with quality indices to assess whether genitalia could be reliable indicators of genetic quality. For this purpose, several single-trait FA measures of functionally important characters (e.g. forearm or finger FA) and one composite FA measure across functional units (finger and mandible FA) were employed. Owing to significant differences between the FA means and variances (see Table 1), forearm and finger length were not combined into a composite wing FA measure, but finger and mandible length were combined to give an ‘average’ asymmetry score. We considered this to be a good measure of composite FA because it was across functional units. However, we also ranked each *N. noctula* for its forearm and finger FA, combined these scores, and then checked

Table 1. Descriptive statistics of signed and unsigned fluctuating asymmetry (FA) (means in mm)

	<i>N</i>	$(L - R)$		$ L - R $	
		Mean \pm SE	var($L - R$)	Mean \pm SE	Slope \pm SE
Forearm	94	0.016 \pm 0.021	1.6341	0.157 \pm 0.014	-0.0041 \pm 0.0142
Finger	87	-0.021 \pm 0.017	0.3693	0.118 \pm 0.011	-0.0025 \pm 0.0265
Tibia	78	0.093 \pm 0.022***	0.2937	0.167 \pm 0.015	0.0041 \pm 0.0346
Toe	75	-0.004 \pm 0.028	0.0780	0.186 \pm 0.018	0.0458 \pm 0.0755
Baculum	69	0.000 \pm 0.006	0.0204	0.038 \pm 0.003	-0.0407 \pm 0.0636
Zygomatic foramen	88	-0.011 \pm 0.011	0.0348	0.078 \pm 0.007	-0.1878 \pm 0.0715*
Mandible	83	0.014 \pm 0.016	0.1106	0.111 \pm 0.010	0.0206 \pm 0.0492
Angular–coronoid	83	0.001 \pm 0.012	0.0437	0.088 \pm 0.007	-0.0916 \pm 0.0595
Condylar–molar	95	0.001 \pm 0.009	0.0326	0.074 \pm 0.005	-0.0146 \pm 0.0616

Slopes are from regressions of $|L - R|$ on trait size. Asterisks indicate significant deviations of $(L - R)$ from zero (*** $P < 0.0001$) and significant regression slopes (* $P < 0.05$), respectively.

whether the wing character scores were correlated with the size of the penis or baculum.

Fluctuating asymmetry was assessed using two-way mixed-model ANOVAs, which allowed the separation of measurement error and directional asymmetry (DA) from non-directional (usually fluctuating) asymmetry (Palmer & Strobeck, 1986). The significance of DA and FA (in the absence of antisymmetry) was tested by the 'side' term and the interaction (side \times individual), respectively, whereas the 'individual' term tested for differences in size or shape among individuals (Palmer, 1994; Merilä & Björklund, 1995). The variation of asymmetry was several orders of magnitude larger than the measurement error (all $P < 0.0001$) and not significantly different from non-DA (for all 'side' terms $P > 0.16$). Signed FA (i.e. left–right, $L - R$) did not deviate from normality (Kolmogorov–Smirnov goodness-of-fit test: condylar–molar: $P = 0.19$; all other traits: $P > 0.84$), and inspection of frequency distribution plots revealed that antisymmetry was unlikely to occur, as there were no bimodal distributions, and skewness and kurtosis were low. Moreover, the means of $(L - R)$ did not deviate significantly from zero in one-sample t -tests, with the exception of tibia length ($t_{77} = 4.262$, $P < 0.0001$; all other traits: $P > 0.2$). Since such deviations are indicative of DA, which may not reflect developmental stability, tibia length was excluded from the statistical analyses of asymmetries. Two measures of FA were calculated, the absolute difference between sides $|L - R|$ and the variance of $(L - R)$ (var($L - R$); Palmer & Strobeck, 1986; Palmer, 1994). As $|L - R|$ values were distributed half-normally, they were normalized by square-root transformations (Sokal & Rohlf, 1995). Furthermore, a regression analysis of $|L - R|$ on trait size yielded a significant relationship for the zygomatic foramen ($r = 0.27$, $t = -2.63$, $P = 0.01$; all other traits: $P > 0.13$), so, for simplicity, this trait was excluded

from final models even though with Bonferroni correction this association was not significant (i.e. we chose to be conservative).

To compare trait size (and FA) with quality measures other than size, body mass was corrected for size by including body length as a covariate in models. This appeared more appropriate than did the use of mass residuals (García-Berthou, 2001). Moreover, month (determined by the collection date of the specimens) and storage duration were included as a factor and covariate, respectively, because body mass can change considerably during the course of the year and could also be affected by the duration of preservation. However, predictor variables were removed from final models by backward elimination if they did not explain statistically significant amounts of variation.

Levels of $|L - R|$ and var($L - R$) among different traits were compared by calculating 95% confidence intervals for $(1 - r)$, with r being the correlation coefficient between sides (Windig & Nylin, 2000). If confidence intervals did not overlap, they were significantly different at the 5% level. Finally, we examined whether levels of asymmetry were related among different traits by performing a series of pairwise Pearson's correlations.

RESULTS

Descriptive statistics of all traits are presented in Table 2. All measurements were highly repeatable, with measurement errors averaging about 1.3% of trait size.

ALLOMETRIC RELATIONSHIPS AND MALE QUALITY

Ordinary least squares regressions of five out of ten variables on body length were statistically significant, and although baculum length was not associated

Table 2. Descriptive statistics and per cent measurement error (% ME) of all traits measured

	<i>N</i>	Mean ± SE	Range	CV	% ME
Body length (mm)	80	86.3 ± 0.47	76.5–96.5	4.85	2.052
Body mass (g)	54	29.62 ± 0.668	21.70–39.59	16.57	1.460
Forearm length (mm)	110	53.01 ± 0.122	50.25–56.80	2.41	0.518
Finger length (mm)	97	19.81 ± 0.062	18.81–21.52	3.07	0.670
Tibia length (mm)	94	19.50 ± 0.056	18.19–20.81	2.78	4.856
Toe length (mm)	75	5.18 ± 0.032	4.50–5.84	5.40	3.316
Penis length (mm)	69	9.29 ± 0.129	6.79–11.39	11.50	1.084
Baculum length (mm)	63	5.78 ± 0.026	5.32–6.20	3.63	1.086
Zygomatic foramen length (mm)	97	5.33 ± 0.019	4.82–5.78	3.50	0.326
Mandible length (mm)	99	14.36 ± 0.033	13.37–15.04	2.32	0.115
Angular–coronoid (mm)	98	5.53 ± 0.021	4.92–6.11	3.78	0.231
Condylar–molar (mm)	99	6.00 ± 0.018	5.51–6.39	3.01	0.251

CV, coefficient of variation.

Table 3. Results of the linear ordinary least squares (OLS) and reduced major axis (RMA) regressions of all skeletal traits on body length

	OLS					RMA	
	<i>N</i>	<i>r</i>	Slope ± SE	<i>t</i> ₀	<i>P</i>	Slope ± SE	<i>t</i> ₁
Forearm length	80	0.180	0.089 ± 0.055	1.618	0.110		
Finger length	66	0.015	0.010 ± 0.081	0.122	0.903		
Tibia length	64	0.423	0.240 ± 0.065	3.675	<0.001	0.567 ± 0.065	–6.629***
Toe length	63	0.150	0.181 ± 0.153	1.181	0.242		
Penis length	60	0.386	0.806 ± 0.253	3.190	0.002	2.086 ± 0.253	4.300***
Baculum length	44	0.220	0.169 ± 0.115	1.464	0.151		
Zygomatic foramen length	74	0.109	0.085 ± 0.092	0.927	0.357		
Mandible length	76	0.346	0.168 ± 0.053	3.173	0.002	0.486 ± 0.053	–9.698***
Angular–coronoid	76	0.227	0.194 ± 0.097	2.003	0.049	0.854 ± 0.097	–1.832
Condylar–molar	77	0.323	0.214 ± 0.073	2.952	0.004	0.663 ± 0.073	–4.640***

RMA slopes are only listed if the OLS regressions are significant; *t*-values from *t*-tests on OLS (*t*₀; H₀: *b* = 0) and on RMA (*t*₁; H₀: *b* = 1), respectively. ****P* < 0.001.

with body length, penis length was (Table 3) (baculum length was also uncorrelated with penis length (*r* = 0.133, *t*₅₂ = 0.96, *P* = 0.34)). Of these significant associations, all except the angular–coronoid remained statistically significant with Bonferroni correction. In subsequent RMA regressions, penis length exhibited strong positive allometry (Table 3, Fig. 1). Among the non-genital traits, angular–coronoid did not deviate from isometry relative to body length (and was not significant after correction for multiple testing in the OLS regressions), whereas all other characters that were significantly associated with body length displayed negative allometry (Table 3). In addition to the positive allometry of penis length, it also displayed higher phenotypic variation than did the other skeletal traits (one-sample *t*-test: *t* = –24.584, *P* < 0.0001;

Table 2). Thus, penis length allometry and variation were consistent with predictions for sexually selected display traits.

To determine whether genital size was associated with either of the quality measures other than size (i.e. FA or mass), a multivariate GLM was performed. Body mass and forearm |*L* – *R*| were used as predictors, with penis and baculum length as dependent variables. We also included body length as a covariate in this analysis even though allometry analysis had already shown a strong body/penis size association because we could then assess mass associations while controlling for body size. Month had no effect and was removed from the final model, but storage duration occasionally had a significant effect and was therefore retained (results not shown as they are not of biolog-

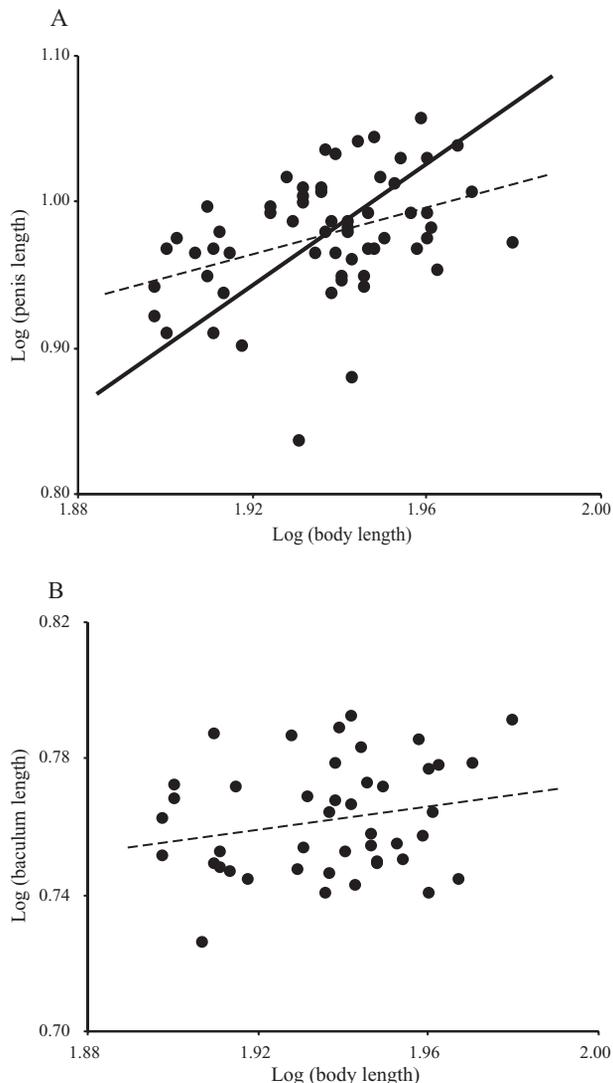


Figure 1. Linear ordinary least squares (OLS; dashed lines) and reduced major axis (RMA; solid lines) regressions on body length for penis (A) and baculum length (B). The RMA slope for baculum length is not drawn because the respective OLS regression was not significant. For slopes and statistics see Table 3.

ical significance, but by including storage duration in the models we could statistically control for storage effects). Body mass had a significant multivariate effect ($F_{2,23} = 5.64$, $P = 0.01$), and univariate tests indicated that this was driven solely by its positive association with penis length ($t = 3.105$, $P = 0.005$; Fig. 2), as mass was not significantly associated with baculum length ($t = -1.537$, $P = 0.137$). There was also a positive association between forearm FA and penis length ($t = 2.351$, $P = 0.027$) but FA was not significantly associated with baculum length ($t = 0.075$, $P = 0.941$). Univariate GLMs used to increase the degrees of free-

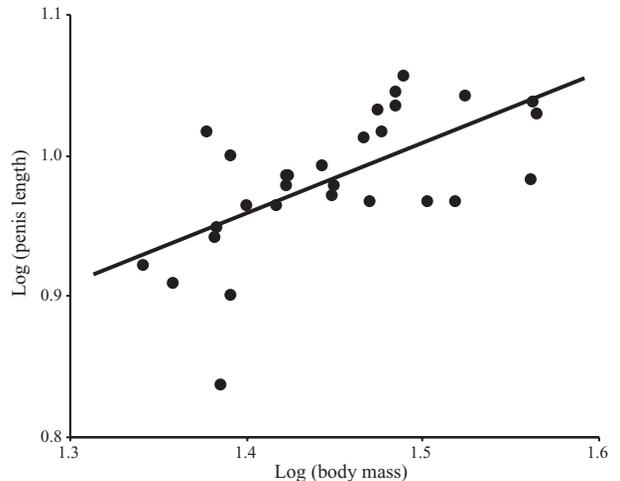


Figure 2. Relationship between penis length and body mass. Although visual inspection may suggest a quadratic relationship between the variables, there is no evidence for this, because in a curve-fit analysis, the linear model had a much higher F -value (17.53 vs. 10.56).

dom (because missing data of penis or baculum length in the multivariate model greatly reduced the sample size), gave the same results, except the association between FA and penis length was no longer significant ($P = 0.38$). Similarly, using other FA measures in multivariate analyses (e.g. finger or composite FA), body mass was always positively associated with penis length (all $P < 0.05$), but never with baculum length (all $P > 0.32$), and FA was never significantly associated with either genital measure (all $P > 0.31$). Similar results were found if we used the ranked scores of wing FA as our composite FA measure: FA was not associated with baculum length, and was positively associated with penis length when analysis was restricted to animals for which we had all data, or it was not associated with either genital trait when we assigned mean rank scores to individuals with missing data. On balance it appeared that penis length correlated with two measures of male quality, size and relative mass, but not with FA, whereas baculum length was never associated with any quality measure.

FLUCTUATING ASYMMETRY

To assess whether FA was trait-specific we performed a series of pairwise Pearson's correlations among all seven traits that exhibited size-independent FA (Table 4). Levels of $|L - R|$ within individuals were only significantly correlated between mandible and angular–coronoid ($r = 0.249$, $P = 0.026$) and between baculum and condylar–molar ($r = -0.268$, $P = 0.048$). However, after correcting for multiple testing, these

Table 4. Pearson's correlation coefficients of fluctuating asymmetry (FA) between different traits within individuals

	Forearm (FO)	Finger (FI)	Toe (TO)	Baculum (BA)	Mandible (MA)	Angular–coronoid (AC)	Condylar–molar (CM)
FO		-0.072 (94)	0.027 (67)	0.073 (59)	0.106 (74)	-0.035 (73)	0.011 (83)
FI	-0.054 (74)		0.047 (57)	0.004 (59)	-0.152 (68)	0.031 (68)	0.189 (74)
TO	0.010 (67)	0.092 (57)		0.026 (47)	0.113 (58)	-0.040 (58)	-0.198 (66)
BA	-0.109 (59)	0.036 (59)	0.053 (47)		-0.123 (51)	0.063 (51)	-0.268 (55)*
MA	-0.205 (74)	-0.081 (68)	-0.107 (58)	0.124 (51)		0.249 (80)**	-0.002 (80)
AC	-0.002 (73)	0.025 (68)	-0.136 (58)	0.120 (51)	0.552 (80)**		-0.147 (81)
CM	0.047 (83)	0.060 (74)	-0.029 (66)	0.065 (55)	0.141 (80)	0.087 (81)	

Above the diagonal are correlations of $|L - R|$, below are correlations of $\text{var}(L - R)$. The abbreviations in the first column refer to the headers of the other columns. Sample sizes in parentheses.

*Significant at $P < 0.05$ before performing a sequential Bonferroni correction.

**Significant at $P < 0.001$ after performing a sequential Bonferroni correction.

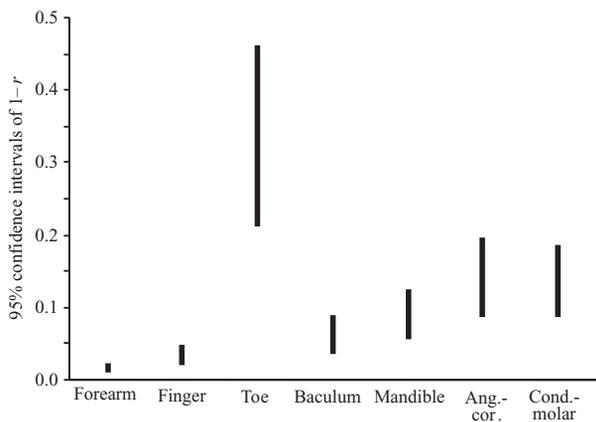


Figure 3. 95% confidence intervals of $1 - r$ values. Traits are significantly different if the bars do not overlap. Ang.-cor., angular–coronoid; Cond.-molar, condylar–molar.

relationships were no longer significant. Using $\text{var}(L - R)$, there was no significant correlation between FA levels, except for that between mandible length and angular–coronoid distance ($r = 0.522$, $P < 0.0001$; all other correlations: r ranging from -0.205 to 0.141 , all $P > 0.079$). Thus FA levels did not generally correlate across traits.

To test for differences in levels of FA across traits, 95% CIs for $1 - r$ were calculated, with r being the correlation coefficient between sides (Windig & Nylin, 2000). The FA of the forearm was significantly lower than it was for all other traits except the finger (i.e. CIs did not overlap), and the upper confidence limit of the finger only overlapped with that of the baculum (Fig. 3). Moreover, toe FA was significantly greater than it was for all other characters, and overall, FA of wing characters (finger & forearm) tended to be lower than it was for other characters.

Finally, we investigated whether there was a relationship between FA and the other quality measures, performing multiple regressions of forearm $|L - R|$ and composite FA (finger and mandible $|L - R|$), respectively, on body length and mass. In both analyses, FA was not significantly associated with the other quality measures (forearm FA: d.f. = 46, $r = 0.288$, $|t| < 1.775$, $P > 0.083$; composite FA: d.f. = 48, $r = 0.088$, $|t| < 0.588$, $P > 0.559$). However, as expected, body mass was significantly correlated with body length (d.f. = 53, $r = 0.693$, $t = 6.939$, $P < 0.0001$).

DISCUSSION

The major result of this study was the strong positive allometry of penis length relative to body length. This contrasted with eight non-genital characters, none of which exhibited positive allometry. Positive allometry is thought to be characteristic of traits under directional sexual selection (Green, 1992; Petrie, 1992; Knell *et al.*, 2004), but is rare in genitalia, which are frequently negatively allometric (Eberhard *et al.*, 1998; reviewed in Hosken & Stockley, 2004), at least in insects. In addition, the phenotypic coefficient of variation (CV) of the penis was 11.5%, about twice the value of most non-genital characters. This is also in accordance with findings that sexually selected traits frequently exhibit a high CV (Price, Schluter & Heckman, 1993; Rowe & Houle, 1996), and the value for the *N. noctula* penis falls well within the range reported for other sexually selected characters across a range of taxa (3.3–90.5%; Pomiankowski & Møller, 1995). To date, studies addressing sexual selection on male genitalia have focused mainly on arthropods (e.g. Waage, 1979; Eberhard *et al.*, 1998; Palestirini, Rolando & Laiolo, 2000; Schmitz, Reinhold & Wagner, 2000; Uhl & Vollrath, 2000; Tatsuta *et al.*, 2001; see also Bamberger & Haase, 2000) or, if focusing on vertebrates,

have typically been across-species comparisons (Patterson, 1983; Dixon, 1987, 1995; Verrell, 1992; Hosken *et al.*, 2001; Stockley, 2002). Consequently, little is known about the allometry and variation of male genitalia within vertebrate species (but see Kelly *et al.*, 2000; Miller & Burton, 2001; Jennions & Kelly, 2002). Nevertheless, to our knowledge, the only study reporting positive allometry of the entire male intromittent organ in a vertebrate (the gonopodium in the guppy *Poecilia reticulata*; Kelly *et al.*, 2000) documented similar values, with allometric slopes of up to 2 and a CV around 8%. However, it is unclear if the gonopodium is subject primarily to natural or to sexual selection (Jennions & Kelly, 2002). Overall, our results are consistent with directional sexual selection on the *N. noctula* penis, but experimental evidence is required to confirm this. In any case, the results strongly contrast with those from many insects, where genitalia frequently show negative allometry and little variation (e.g. Eberhard *et al.*, 1998; Uhl & Vollrath, 2000). It is unclear why such differences exist, but one obvious difference is that insect genitalia are (at least partly) sclerotized, while the mammalian penis is a less static structure. Further studies are required to see if the mammalian penis is typically positively allometric, but the limited evidence available suggests that selection on the vertebrate intromittent organ differs somewhat from that on the more frequently studied insect aedeagus.

It has also been suggested that genital size could be an honest indicator of male genetic quality (Eberhard, 1985; Miller & Burton, 2001; Simmons, 2001). If so, genital size should correlate with some measure of male quality. The positive associations between penis length and size-corrected body mass and body length indicate that the penis indeed reflects some phenotypic measures of male quality. How these phenotypic quality measures relate to genetic quality was not assessed, but the measures we employed are those typically used in evolutionary ecology (Blanckenhorn & Hosken, 2003). Additionally, body size, and morphological traits in general, are typically highly heritable (Roff, 1997). That the penis reflects male (phenotypic and possibly genetic) quality may be important in *N. noctula* as copulation occurs during torpor when females cannot actively assess mates. Nevertheless, we cannot distinguish between mechanisms of sexual selection (sexual conflict, sperm competition or cryptic female choice) with our data, and indeed this is something that is generally very difficult to do (see Simmons, 2001; Eberhard, 2004; Hosken & Stockley, 2004). However, as with the seal study of Miller & Burton (2001), female *N. noctula* can potentially obtain good-genes benefits via cryptic female choice (in the broadest sense) based on penis size.

In contrast to penis length, baculum length did not scale with body length, and its CV (3.6%) was in the range of the non-genital, skeletal traits (1.99–5.40%). Additionally, it was not related to any quality measure. If directional sexual selection is associated with positive allometry and high phenotypic variation, there is no evidence that the baculum of *N. noctula* is directionally selected. These findings complement those of Hosken *et al.* (2001), who found no indication of sexual selection on the length of the bat os penis in an across-species study, although not all aspects of sexual selection have been investigated. In contrast, the baculum of harp seals is positively allometric relative to body size, and thought to be an honest indicator of male size (Miller & Burton, 2001). Similarly, across carnivores, there is evidence that the baculum is sexually selected (Dixon, 1995). Across bats, however, the reasons for baculum diversity remain unclear. Nonetheless, the baculum data presented here resemble patterns documented for insect genitalia, although the exact function of the baculum has not been established.

One frequently used measure of genetic quality is FA (Palmer & Strobeck, 1986; Møller, 1990; Møller & Swaddle, 1997; but see Blanckenhorn & Hosken, 2003). If FA were a reliable quality measure, we would expect the FA of different traits within individuals to be positively associated, since all traits are under similar genetic control and should be exposed to similar levels of stress during development (Leamy, 1993; Lens & Van Dongen, 1999; but see Whitlock, 1996). However, there was no general concordance of FA across traits, with only one of many comparisons being statistically significant. These results agree with many studies of individual-level FA (e.g. Leamy, 1984; Kimball, Ligon & Merola-Zwartjes, 1997; Hosken *et al.*, 2000), and few investigations (e.g. Lens & Van Dongen, 1999) have found evidence for organism-wide asymmetry. Consequently, FA appears generally to be trait-specific. FA was also not associated with either of the other two quality measures, and frequently neither the genetic basis of FA nor its value as a broad reflector of stress and genetic quality are clear (Bjorksten *et al.*, 2000; Hosken *et al.*, 2000; Fuller & Houle, 2002; Martin & Hosken, 2002; Blanckenhorn & Hosken, 2003; Hosken, Garner & Blanckenhorn, 2003). Nevertheless, Palmer & Strobeck (1986) suggested that levels of trait asymmetry should decrease with the increasing functional importance of characters. Gummer & Brigham (1995) supported this hypothesis in their study on little brown bats (*Myotis lucifugus*), finding higher variation of FA in hindlimbs than in wings. In our study, traits considered to be more functionally important (e.g. wing bones) also tended to be more symmetrical compared with less important traits (e.g. toes; see Fig. 3).

In conclusion, the penis of *N. noctula* exhibited strong positive allometry relative to body length, high phenotypic variation and positive associations with most quality measures. These attributes are typical of characters subject to directional sexual selection, and the penis seems to be a reliable indicator of male quality. Our results contrast starkly with investigations of insect genitalia, but the reasons for these differences remain unresolved.

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