Electronic Supplementary Material

The baculum affects paternity success of first but not second males in house mouse sperm competition

Contents

1. Detailed baculum measurement protocol

1.1. Baculum measurements

After euthanasia of the male, the penis was immediately dissected out. For baculum preparation, most penis tissue was carefully removed using dissection tools (partially under a dissection microscope). The remaining tissue was soaked in an Eppendorf tube in 1 ml of 0.05 g/ml KOH for 19-22h, after which the small distal tip of the bone and all remaining tissue was removed (modified after Ramm et al. 2010; Stockley et al. 2013; Simmons and Firman 2014). The bacula were then stored at 4°C in Eppendorf tubes containing 1ml of 100% Ethanol. Bacula were photographed through a microscope (Nikon SMZ 745T; Nikon GmbH, Düsseldorf, Germany) against a black background, alongside a micrometer scale for size calibration (Fig 1A). Photos were taken at 45x magnification with a Basler acA1920-40uc camera and the program Pylon Viewer (Basler AG; Basler AG, Ahrensburg, Germany). Each baculum was photographed from both sides. The side with a rather concave baculum base was defined 'upper' side and the rather convex side 'down' side. Repeatability was assessed comparing 'upper' and 'down' side measurements. All pictures and measurements were taken blindly with respect to male ID and treatment by the first author. Repeatability was measured by intra-class correlation coefficients (ICC; see Table S2) using R package 'ICC' (Wolak et al. 2012). We refer to the direct measurements (area, width and length) as 'size' and to the geometric morphometrics parameters as 'shape'; we appreciate that neither of them purely reflects size or shape. For example, direct measurements are influenced by shape, and the measure of centroid size in the geometric morphometrics analysis is a measure of size.

1.2. Baculum size: Direct measurements

The bacula were measured using ImageJ (v1.49; Schneider et al. 2012) after size calibration. The picture was first transformed to black and white applying a threshold. After transformation, the area was measured automatically using the 'wand' tool provided by ImageJ. The threshold was set manually for each image due to slightly varying light conditions and baculum brightness, but there was no correlation between threshold and measured baculum area (data not shown). All other measures were taken on the original picture. The total length, base and shaft width were measured on a straight line by hand (Figure 1A). Base width represents the width at the widest part of the base of the baculum, and shaft width the width at the narrowest part of the shaft (Figure 1A). Baculum measurements were repeatable, with ICC larger than 0.68. Paired t-tests were used to compare both measures (Table S1). The sides were not significantly different (not shown) and the means of both measures were used in all subsequent analyses concerning size measurements.

1.3. Baculum shape: Geometric morphometrics

In addition to the direct measurements of the baculum extracted using *ImageJ*, its shape was quantified morphometrically using *tpsDig2* (v2.31; Rohlf 2015). Two fixed and 38 semi-sliding landmarks were used to outline the baculum (modified after Simmons and Firman 2014; André et al. 2018). Fixed landmarks were placed on the most proximal and distal positions of the baculum (Figure 1B; Figure S1). The 38 semi-sliding landmarks were equally spaced on the periphery of the baculum and set as sliding (i.e. moving between the neighboring landmarks) in the analysis program. Analysis files were created with the pictures for measuring and appending curves to landmarks using *tpsUtil* (v1.76; Rohlf 2015). Centroid size and relative warps were extracted using *tpsRelw* (v1.69; Rohlf 2015). Centroid size is a measure that represents size independently from shape in the absence of allometry (i.e. centroid size is only correlated with shape if they change together; Zelditch et al. 2004). The relative warps (RW) express the variation of shape relative to the consensus configuration across all bacula (Zelditch et al. 2004). 'Upper' and 'down' sides of the baculum were analyzed separately, because RW appeared to be different for the sides and the flipped orientation of the baculum might influence shape measures. Nevertheless, because results for 'upper' and 'down' side were qualitatively (and often also quantitatively) similar, only the results for the 'upper' side are reported for brevity. Additionally, the repeatabilities for centroid size and relative warp scores were analyzed by remeasuring the same set of pictures twice (Table S2).

Shape description of RW scores (i.e. what kind of alteration in shape is described by the RW) was assessed by plotting the extremes and vector-plots of the RW using *tpsRelw* (v1.69; Rohlf 2015; Figures S2 & S3). The first two relative warps are presented in the subsequent analyses. These explain together over 60% of the variation in shape (Table S3). Lower scores for relative warp score 1 (RW1) indicate that the baculum has a 'stretched' base (and dorsal base-end) while higher scores mean a longitudinally 'compressed' base (and dorsal base-end; Figure S2). Thus, the base is relatively wider but shorter for larger RW1 values and narrower but longer for negative values of RW1. Larger RW1 scores also describe shorter bacula, due to the 'compressed' base. Relative warp score 2 describes variation in the width of the base of the baculum. Negative scores mean a wider base, while positive scores mean a narrower base (Figure S3). Larger RW2 scores also describe slightly longer bacula.

2. Baculum measurements: variation, repeatability, variance inflation factors

The following section of figures presents what kind of shape variation the relative warp scores describe. For the different relative warps, we present the consensus configuration, negative/positive approximate minimum/maximum of warp scores and an extreme of negative/positive 0.1.

Figure S1: Plot of all individual landmark positions of all baculum samples. 40 landmarks with two fixed and 38 semi-sliding.

Relative warp score 1 mainly describes variation in the base of the baculum, meaning that the baculum has a "stretched" base (and dorsal base-end) for lower scores and a "compressed" base (and dorsal base-end) for higher scores (see figures S2). The base is wider for high RW1 values and narrower, while longer, for negative values of RW 1.

Figure S2: Relative warp score 1. Left to right RW: -0.1; -0.027; 0.041; 0.1. Change in RW represented by vectors for outer figures.

Relative warp score 2 describes variation in the width of the base of the bacula. Negative scores mean a wider base, while positive scores mean a narrower base (see figure S3). Large RW 2 scores also describe slightly longer bacula (and vice versa).

Figure S3: Relative warp score 2. Left to right RW: -0.1; -0.037; 0.029; 0.1. Change in RW represented by vectors for outer figures.

Table S2: Interclass correlation coefficients (ICC) of baculum measurements, with lower/upper confidence interval, N (sample size), number of measurements per individual (if unbalanced calculated after Lessells & Boag (1987), the within individual variance and among individual variance.

Table S3: Singular values and percentage of variance explained for the first two relative warps.

Table S4: Variance inflation factors of (a) baculum size and (b) baculum shape models. Binomial GLMMs with OLRE. Model specified in left column. Additional rows refer to recalculated VIF after removals. NA means variable dropped due to variance inflation >3.

3. Fitness effects: Interactions between baculum morphology and genotype

3.1. First male – Size

a)

Table S5: Results of beta-binomial GLMM for the proportion of embryos sired by the first male (weighted by litter size) including body mass and genotype as covariates. First male baculum size measures as predictors, including interactions with genotype. resid. df=30.

3.2. First male – Morphometrics

Table S6: Results of beta-binomial GLMM for the proportion of embryos sired by the first male (weighted by litter size) including body mass and genotype as covariates. First male morphometric baculum measures as predictors including interactions with genotype. resid. df=28.

3.3. Second male – Size

Table S7: Results of beta-binomial GLMM for the proportion of embryos sired by the second male (weighted by litter size) including body mass and genotype as covariates. Second male baculum size measures as predictors, including interactions with genotype. resid. df=31.

3.4. Second male – Morphometrics

Table S8: Results of beta-binomial GLMM for the proportion of embryos sired by the second male (weighted by litter size) including body mass and genotype as covariates. Second male morphometric baculum measures as predictors, including interactions with genotype. resid. df=31.

4. Fitness effects: Interactions between baculum morphology and plug removal

4.1. First male – Size

Table S9: Results of beta-binomial GLMM for the proportion of embryos sired by the first male (weighted by litter size) including body mass and genotype as covariates. First male baculum size measures as predictors, including interactions with plug removal treatment. resid. df=29.

4.2. First male – Morphometrics

Table S10: Results of beta-binomial GLMM for the proportion of embryos sired by the first-to-mate male (weighted by litter size) including body mass and genotype as covariates. First male morphometric baculum measures as predictors, including interactions with plug removal treatment. resid. df=27.

4.3. Second male – Size

Table S11: Results of beta-binomial GLMM for the proportion of embryos sired by the second male (weighted by litter size) including body mass and genotype as covariates. Second male baculum size measures as predictors, including interactions with plug removal treatment. resid. df=30.

4.4. Second male – Morphometrics

See table 2 in main text.

5. Copulatory behaviour and the baculum

5.1. Morphometrics for larger dataset

Table S12: Singular values and percent explained for relative warps of larger dataset for behaviour analysis.

Figure S4: Relative warp score 1 of larger dataset for behaviour analysis. Left to right RW: -0.1; -0.027; 0.039; 0.1. Change in RW represented by vectors for outer figures.

Relative warp score 1 means that the baculum has a "stretched" base (and dorsal base-end) for lower scores and a "compressed" base (and dorsal base-end) for higher scores (see figures S5). The base is wider for high RW1 values and narrower, while longer, for negative values of RW 1.

Figure S5: Relative warp score 2 of larger dataset for behaviour analysis. Left to right RW: -0.1; -0.035; 0.030; 0.1. Change in RW represented by vectors for outer figures.

Relative warp score 2 describes variation in the wideness of the base of the bacula. Negative scores mean a wider base, while positive scores mean a smaller base (see figure S6). Large RW 2 scores also describe slightly longer bacula.

5.2. First male

Table S14: Results of linear mixed model for behavioural measurements (specified in 'model') by the first male including body mass as covariates. Morphometric baculum measures as predictors.

5.3. Second male

Table S15: Results of linear mixed model for behavioural measurements (specified in 'model') by the second male including body mass and as covariates. Size baculum measures as predictors.

Table S16: Results of linear mixed model for behavioural measurements (specified in 'model') by the second male including body mass as covariates. Morphometric baculum measures as predictors.

6. No morphological differences between +/+ and +/*t.*

Table S17: Unpaired, two sided 'Welch Two Sample' t-tests for differences between +/+ and +/*t* males in various morphological baculum features. p-value 'FDR' adjusted.

7. No influence of testes and seminal vesicle weight.

Table S18: Results of generalised linear mixed model of family beta-binomial for the proportion of embryos sired by the first male including body mass, testes weight, seminal vesicle weight and genotype as covariates. Model weight by the total number of embryos per female. df=36.

8. Model selection via AICc

Table S19: Results of generalized linear mixed models of family beta-binomial for the proportion of embryos sired by the first male (weighted by litter size) including body mass and genotype as covariates. Size and morphometric baculum measures in separate models as predictors. Models were compared by AICc, and the models with the lowest AICc are presented here (for full models see Table 1). M1/M2 = first/second-to-mate male.

8.1. Genotype interaction: Second male – Morphometrics

Table S20: Results of generalized linear mixed model of family beta-binomial for the proportion of embryos sired by the second-to-mate male (weighted by litter size) including body mass and genotype as covariates. Morphometric baculum measures as predictors including plug removal treatment interactions. df=37. Models were compared by AICc, and the models with the lowest AICc are presented here (for full models see Table 2).

Figure S6: Proportion of embryos sired by the first male plotted against the centered and scaled base width of the first-to-mate male. Regression represents predicted values (with 95% confidence intervals in grey) from beta-binomial model.

9. References

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