**Electronic Supplementary Materials**

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

Gonçalo I. André \*, Renée C. Firman, Leigh W. Simmons

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

Correspondence: goncalo.igrejaandre@research.uwa.edu.au

This file contains, supplementary figures (Figure S1, S2, S3 and S4) and supplementary tables (Table S1, S2, S3, S4, S5 and S6)

**Figure S1.** Variation in baculum shape of second-generation laboratory reared house mice derived from the extremes of baculum shape (Wide/Narrow). *(a)* Mean (± 95% CI) score of the first relative warp (RW1). *(b)* Mean (± 95% CI) score of the second relative warp (RW2). Baculum shape thin-plate splines: left– wide background, right– narrow background.

*(a)*



*(b)*



**Figure S2.** Variation in baculum shape of third-generation laboratory reared house mice descended from families from the extremes of baculum shape (Wide/Narrow). *(a)* Mean (± 95% CI) score of the first relative warp (RW1). *(b)* Mean (± 95% CI) score of the second relative warp (RW2). Baculum shape thin-plate splines: left– wide background, right– narrow background.

*(a)*





*(b)*

**Table S1.** Linear mixed model (LMM) of baculum shape of F2 and F3 house mice descended from families from the extremes of baculum shape. (a) Second generation (n= 104); (b) Third generation (n=214). Values in italics are significant at p < 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **fixed effects**  | **estimate ± (se)** | **type II, Wald χ2** | ***p*** | **random effects**  | **variance**  | **±sd** |
| 1. *2nd Generation*
 |  |  |  |  |  |  |
| RW1  |  |  |  |  |  |  |
| intercept |  -0.071 (0.042) |  |  | family | <0.0001 | 0.0006 |
| extreme background |  -0.013 (0.004) | 8.37 | **0.003** |  |  |  |
| body weight | 0.027 (0.015) | 3.43 | 0.06 |  |  |  |
| RW2 |  |  |  |  |  |  |
| intercept | 0.031 (0.036) |  |  | family | <0.0001 | 0.0027 |
| extreme background | 0.002 (0.003) | 0.27 | 0.61 |  |  |  |
| body weight |  -0.012 (0.013) | 0.83 | 0.36 |  |  |  |
| Centroid Size |  |  |  |  |  |  |
| intercept | 0.423 (1.209) |  |  | family | 0.0502 | 0.2241 |
| extreme background |  -0.084 (0.149) | 0.31 | 0.57 |  |  |  |
| body weight |  -0.135 (0.425) | 0.11 | 0.75 |  |  |  |
|  |  |  |  |  |  |  |
|  *(b) 3rd Generation*  |  |  |  |  |  |  |
| RW1  |  |  |  |  |  |  |
| intercept |  -0.058 (0.032) |  |  | family | <0.0001 | 0.0051 |
| extreme background |  -0.004 (0.003) | 1.92 | 0.16 |  |  |  |
| body weight | 0.021 (0.011) | 3.55 | 0.06 |  |  |  |
| RW2 |  |  |  |  |  |  |
| intercept | 0.039 (0.025) |  |  | family | <0.0001 | 0.0069 |
| extreme background | 0.014 (0.003) | 14.69 | **0.0001** |  |  |  |
| body weight |  -0.016 (0.008) | 3.48 | 0.06 |  |  |  |
| Centroid Size |  |  |  |  |  |  |
| intercept |  -3.382 (0.814) |  |  | family | 0.0581 | 0.2411 |
| extreme background | 0.053 (0.121) | 0.19 | 0.66 |  |  |  |
| body weight | 1.182 (0.289) | 16.64 | **<0.001** |   |   |   |

**Figure S3.** Mating design. a) Monandrous matings – Males and females from different families and from similar or opposite baculum extreme backgrounds (wide and narrow) were coupled in a multifactorial way (*n* = 77 mating pairs); b) Polyandrous matings – Females from different baculum extreme backgrounds (wide and narrow) were coupled sequentially with males from different baculum extreme backgrounds in a multifactorial way ( *n* = 74 mating trios).

Wide

Narrow

Wide

Narrow

Narrow

Wide

Wide

Narrow

Narrow

Narrow

Wide

Wide

**X**

**X**

**X**

**X**

**X**

**X**

**X**

**X**

1st mating

2nd mating

*b)*

Narrow

Wide

Narrow

Narrow

Wide

Wide

Narrow

Wide

**X**

**X**

**X**

**X**

*a)*

**Table S2.** Microsatellite loci used to assign embryo parentage. Microsatellite loci were divided into two multiplexes (a & b).

|  |  |  |
| --- | --- | --- |
| Locus Name  | Left (5' - 3') | Right (5'-3') |
| *(a) Multiplex 1* |  |
| **D1Mit17** | GTGTCTGCCTTTGCACCTTT | CTGCTGTCTTTCCATCCACA |
| **D10Mit14** | AGAGGGGACAAGGAGAGACC | AAGGTTTGGGTTCAGTTCCCAG |
| **D11Mit4** | CAGTGGGTCATCAGTACAGCA | AAGCCAGCCCAGTCTTCATA |
| **D14Mit132** | GAACAGCACCATCCACACACC | GTGGGGTTATATGCAGATAC |
| **D18Mit17** | TCAGGCAGATTCCAAGCAG | CTGTGGGTAGCCCAAGTCAT |
| *(b) Multiplex 2*  |  |
| **D2Mit1** | CTTTTTCGTATGTGGTGGGG | AACATTGGGCCTCTATGCAC |
| **D4Mit22** | GCAGTTAAACTGTACTTTCTG | CTCAGACATGATTTTTTCCAA |
| **D6Mit138** | CAAAGAAAGCATTTCAAGAC | GCTCTTATTAATGAAGAAGA |
| **D13Mit1** | GTCTGTTTGATTCCTGACCTCC | TCAACTCTTCTGTAAACCAGATG |
| **D15Mit13** | GGAGACAAAAATGAACTCCTGG | TTGTAAGACAAGCATAGCTCAAC |

**Figure S4.** Landmark placement on images of the baculum of the house mouse, *Mus musculus domesticus.* The placement of 40 landmarks are shown, 4 fixed landmarks (white, numbers: 1, 7, 21 and 35) and 36 semi-sliding landmarks (red) placed evenly between fixed landmarks around its periphery.

**1**

**7**

**21**

**35**

Landmark placement details: For the baculum, fixed landmarks demarked the most lateral, anterior and posterior positions of the baculum, homologous structures across all specimens. Sliding semi-landmarks were spaced evenly between the fixed landmarks (Zelditch et al. 2012).

**Table S3**. Repeatability analysis. (a) Second generation, (b) Third generation. We used intra-class correlation coefficient analysis (ICC) with the one-way random effects model to estimate the reliability of measurement for landmark placement. Values in italics are significant at p < 0.05.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   | ***n*** | **ICC** | **Confidence Interval**  | **F** | **p** |
| *(a)2nd Generation* |  |  |  |  |  |
| RW1 | 21 | 0.992 | 0.982 - 0.997 | 266 | <0.0001 |
| RW2 | 21 | 0.982 | 0.958 - 0.993 | 113 | <0.0001 |
| Centroid Size  | 21 | 0.996 | 0.991 - 0.998 | 537 | <0.0001 |
|  |  |  |  |  |  |
| *(b) 3rd Generation*  |  |  |  |  |  |
| RW1 | 42 | 0.974 | 0.953 - 0.986 | 76.2 | <0.0001 |
| RW2 | 42 | 0.979 | 0.961 - 0.989 | 93.6 | <0.0001 |
| Centroid Size  | 42 | 0.998 | 0.996 - 0.999 | 915 | <0.0001 |

**Table S4.** General Linear Mixed Model (GLMM) of the effect of baculum morphology and its interaction with female genetic background on the number of embryos. Values in italics are significant at p < 0.05.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **fixed effects**  | **estimate ± (se)** | **type III, Wald χ2**  | **p** | **random effects**  | **variance**  | **±sd** |
| intercept | 2.059 (0.059) |  |  | ♂ Family | <0.0001 | <0.0001 |
| RW1  | 0.408 (2.725) | 0.022 | 0.88 | ♀ Family | <0.0001 | <0.0001 |
| RW2 | 1.447 (3.493) | 0.172 | 0.68 |  |  |  |
| Baculum Size | 0.052 (0.109) | 0.228 | 0.63 |  |  |  |
| ♀ Extreme |  -0.043 (0.083) | 0.267 | 0.61 |  |  |  |
| RW1 × ♀Extreme |  -0.275 (4.338) | 0.004 | 0.95 |  |  |  |
| RW2 × ♀Extreme |  -5.975 (5.750) | 1.079 | 0.29 |  |  |  |
| Baculum Size × ♀Extreme |  -0.024 (0.171) | 0.019 | 0.88 |   |   |   |

 |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| **Table S5**. General Linear Mixed Model (GLMM) of the effect of baculum morphology and its interaction with female genetic background on embryo viability. Values in italics are significant at p < 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **fixed effects**  | **estimate ± (se)** | **type III, Wald χ2**  | **p** | **random effects**  | **variance**  | **±sd** |
| intercept | 2.874 (0.318) |  |  | ♂ Family | <0.0001 | <0.0001 |
| RW1  | 4.631 (11.741) | 0.156 | 0.69 | ♀ Family | 0.227 | 0.477 |
| RW2 |  0.923 (14.699) | 0.004 | 0.95 |  |  |  |
| Baculum Size | 0.885 (0.511) | 3.006 | 0.08 |  |  |  |
| ♀ Extreme |  -0.354 (0.347) | 1.044 | 0.18 |  |  |  |
| RW1 × ♀Extreme |  -10.11 (17.11) | 0.349 | 0.54 |  |  |  |
| RW2 × ♀Extreme | 6.531 (21.766) | 0.090 | 0.62 |  |  |  |
| Baculum Size × ♀Extreme |  -0.272 (0.707) | 0.148 | 0.44 |   |   |   |

  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

**Table S6.** General Linear Mixed Model (GLMM) of the effect of baculum shape of the first male relative to the second male and its interaction with the female’s genetic background on second male parentage for the reduced sample of mating trios in which paternity could be assigned to 80% or more of the embryos (males = 132; females = 66; total embryos = 491). Values in italics are significant at p < 0.05.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **fixed effects**  | **estimate ± (se)** | **type III, Wald χ2** | ***p*** | **random effects**  | **variance**  | **± s.d.** |
| intercept |  -0.588 (0.344) |   |   | 1st ♂ Family | 0.782 | 0.884 |
| RW1 |  -3.188 (8.345) | 0.833 | 0.36 | 2nd ♂ Family | 0.674 | 0.812 |
| RW2 | 7.541 (8.266) | 0.456 | 0.49 | ♀ Family | <0.0001 | <0.0001 |
| Baculum Size |  1.163 (2.001) | 0.877 | 0.35 |  |  |  |
| ♀ Extreme | 0.611 (0.288) | 3.272 | 0.07 |  |  |  |
| RW1 × ♀ Extreme |  -3.871 (10.290) | 0.141 | 0.71 |  |  |  |
| RW2 × ♀ Extreme |  -26.17 (12.847) | 4.151 | **0.04** |  |  |  |
| Baculum Size × ♀ Extreme | 0.663 (3.068) | 0.046 | 0.83 |   |   |   |