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Supplemental Data

Evolutionary Response to Sexual

Selection in Male Genital Morphology

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Supplemental Experimental Procedures

Genital Measurements

We dissected the aedeagus from the body cavity, and placed it onto a smear of Vaseline on a microscope slide. The aedeagus was oriented to provide a lateral view before an image was captured using the Optimas Image Analysis package (Media Cybernetics Silver Spring, MD). We placed 8 landmarks (A-F) at defined locations around the periphery of the aedeagus image (see Figure 1a in the main text) and measured the linear distance between each landmark. The different measurements were highly correlated, and we used Principal Components (PC) Analysis to summarise the variation in the multivariate data set and reduce it to fewer uncorrelated variables. We retained only those PCs that had eigenvalues greater than 1.

The 5 genital sclerites were dissected from the phallobase of the aedeagus, and the connective tissue between them was macerated in 10% KOH (30 min), and cleared in 50% aqueous lactic acid (30 min) before sclerites were mounted on slides using Hoyers solution. The linear distances between defined landmarks on each sclerite were measured (see Figure 2 in the main text). We conducted a PCA for each sclerite, again retaining only those PCs with eigenvalues greater than 1. Sclerite 1 had only a single measurement, so we calculated a Z-score of that measurement so that its statistical properties were the same as the PC scores for sclerites 2-5. Data on the relationship between fertilization success and genital sclerite morphology came from 3 previous studies in which a variety of sclerite measurements had been taken [1-3]. We therefore used only those measurements that were common to all 3 studies (sclerite 1 A-B and outline; Sclerite 2 A-B, area, and outline; Sclerite 3 A-B, area, and outline; Sclerite 4 B-D, area, and outline; and Sclerite 5 A-B, A-C, area and outline). For each sclerite the PCA reduced the variation to a single PC with an eigenvalue greater than 1.

More sophisticated morphometric techniques such as geometric morphometric analysis [4] are increasingly being used to quantify size and shape variation. We chose the simple landmark based method here for three reasons. Most importantly, our previous studies of the influence of genital sclerites on sperm competitive success [1] and our analysis of quantitative genetic variation in genital sclerites used this method [5], and we wanted to draw direct comparisons with these studies. Secondly, our previous studies [1], and our results here, show that this technique captures biologically relevant variation; landmark based measures of genital size and shape predict variation in male fitness. Finally, studies of waterstrider genitalia that compared simple landmark based analysis with geometric morphometric techniques show that both methods capture equivalent amounts of phenotypic and genetic variation in male genital morphology [6].

Supplemental References

1. House, C.M., and Simmons, L.W. (2003). Genital morphology and fertilization success in the dung beetle *Onthophagus taurus*: an example of sexually selected male genitalia. Proc. R. Soc. Lond. B *270*, 447-455.
2. House, C.M., and Simmons, L.W. (2005). Relative influence of male and female genital morphology on paternity in the dung beetle *Onthophagus taurus*. Behav. Ecol. *16*, 889-897.
3. House, C.M., and Simmons, L.W. (2006). Offensive and defensive sperm competition roles in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). Behav. Ecol. Sociobiol. *60*, 131-136.
4. Zelditch, M.L., Swiderski, D.L., Sheets, H.D., and Fink, W.L. (2004). Geometric Morphometrics for Biologists. A Primer (London: Elsevier Academic Press).
5. House, C.M., and Simmons, L.W. (2005). The evolution of male genitalia: patterns of genetic variation and covariation in the genital sclerites of the dung beetle *Onthophagus taurus*. J. Evol. Biol. *18*, 1281-1292.
6. Arnqvist, G., and Thornhill, R. (1998). Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition dependence of genital and non-genital morphology in water striders (Heteroptera: Gerridae: Insecta). Genet. Res., Camb. *71*, 193-212.

Table S1. Principal components analysis of linear measurements of the aedeagus of males derived from 3 replicate lines of *Onthophagus taurus* evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Trait	PC1	PC2	PC3
Eigenvalue	2.799	1.486	1.113
% variance	34.99	18.58	13.92
A-B	0.168	–0.565	0.139
B-C	0.217	0.654	–0.048
C-G	0.523	–0.028	0.349
D-E	0.180	–0.073	–0.669
E-F	0.447	–0.088	–0.115
F-G	0.320	0.340	–0.296
G-D	0.542	–0.056	0.269
G-A	–0.153	0.356	0.486

Table S2. ANOVAs in aedeagus morphology of male *Onthophagus taurus* from 3 replicate lines evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Trait Source	SS	df	MS	F	P
Aedeagus PC1					
Selection history	9.60	1	9.60	1.46	0.292
Line[selection history]	26.50	4	6.63	2.48	0.046
Error	494.39	185			
Aedeagus PC2					
Selection history	9.43	1	9.43	17.62	0.011
Line[selection history]	2.08	4	0.52	0.36	0.839
Error	269.57	185			
Aedeagus PC3					
Selection history	7.90	1	7.90	2.44	0.192
Line[selection history]	13.07	4	3.27	3.17	0.015
Error	190.53	185			

SS, sums of squares; df, degrees of freedom; MS, mean squares; F, variance ratio; P, probability

Table S3. ANOVA in the pronotum width of male *Onthophagus taurus* from 3 replicate lines evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Source	SS	df	MS	F	P
Selection history	0.241	1	0.241	0.897	0.397
Line[selection history]	1.085	4	0.271	5.039	<0.001
Error	10.440	194			

SS, sums of squares; df, degrees of freedom; MS, mean squares; F, variance ratio; P, probability

Table S4. Principal components analysis of linear measurements of the genital sclerites of males derived from 3 replicate lines of *Onthophagus taurus* evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Variable	Sclerite 2		Sclerite 3	Sclerite 4	Sclerite 5	
	PC1	PC2	PC1	PC1	PC1	PC2
Eigenvalue	1.483	1.133	1.657	1.213	2.331	1.074
% variance	49.44	37.77	55.24	60.63	58.27	26.86
A-B	0.690	–0.353	0.591	-	0.235	0.871
A-C	0.072	0.903	0.503	0.707	0.575	0.064
A-D	-	-	-	-	0.599	0.009
A-E	-	-	-	-	0.506	–0.487
B-C	0.720	0.247	0.631	-	-	-
B-D	-	-	-	–0.707	-	-

Table S5. ANOVAs in genital sclerite morphology of male *Onthophagus taurus* from 3 replicate lines evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Trait Source	SS	df	MS	F	P
Sclerite 1					
Selection history	0.002	1	0.002	6.36	0.062
Line[selection history]	0.001	4	0.000	0.30	0.876
Error	0.173	162			
Sclerite 2 PC1					
Selection history	3.02	1	3.02	2.14	0.146
Line[selection history]	15.17	4	3.79	2.69	0.034
Error	217.38	154			
Sclerite 2 PC2					
Selection history	1.11	1	1.11	2.37	0.197
Line[selection history]	1.87	4	0.47	0.41	0.804
Error	177.14	154			
Sclerite 3					
Selection history	1.25	1	1.25	0.17	0.703
Line[selection history]	29.97	4	7.49	4.94	<0.001
Error	241.05	159			
Sclerite 4					
Selection history	0.51	1	0.51	0.17	0.700
Line[selection history]	11.91	4	2.98	2.54	0.042
Error	190.20	162			
Sclerite 5 PC1					
Selection history	0.55	1	0.55	0.12	0.751
Line[selection history]	19.14	4	4.79	2.10	0.083
Error	346.21	152			
Sclerite 5 PC2					
Selection history	0.63	1	0.63	0.31	0.609
Line[selection history]	8.26	4	2.06	1.97	0.102
Error	159.53	152			

SS, sums of squares; df, degrees of freedom; MS, mean squares; F, variance ratio; P, probability

Table S6. Principal components analysis of linear measurements of the aedeagus of male *Onthophagus taurus* used to estimate the form and strength of sexual selection via male mating success

Trait	PC1	PC2	PC3
Eigenvalue	2.260	1.636	1.309
% variance	28.25	20.45	16.37
A-B	–0.067	–0.534	0.250
B-C	0.186	–0.496	0.442
C-G	0.608	–0.049	0.067
D-E	–0.152	–0.192	0.392
E-F	0.199	0.313	0.493
F-G	–0.345	0.403	0.352
G-D	0.565	0.162	0.184
G-A	–0.300	–0.379	0.427

Table S7. Standardized linear (β) and non-linear (γ) sexual selection gradients (±SE) for principal component descriptors of aedeagus morphology and body size.

	β	γ
PC1	0.022±0.050	–0.054±0.030
PC2	–0.101±0.046*	0.014±0.023
PC3	–0.073±0.059	0.044±0.037
Pronotum	–0.300±0.063**	–0.056±0.044

*p<0.05, **p<0.001

Table S8. First principal components from separate analyses of the measurements of 5 genital sclerites (SC1-SC5) of male *Onthophagus taurus* mating in the defensive role of first male in sperm competition trials

Trait	SC1	SC2	SC3	SC4	SC5
Eigenvalue	1.180	2.121	1.838	2.493	2.276
% variance	59.02	70.70	61.27	83.09	56.89
A-B	0.768	0.725	0.452		0.376
A-C					0.831
B-D				0.868	
area		0.845	0.860	0.934	0.901
outline	0.768	0.939	0.946	0.931	0.795

Table S9. Standardized linear (β), and matrix of quadratic and correlational selection gradients (γ) acting on the five genital sclerites of male *Onthophagus taurus* mating in the defensive role of first male

		γ				
	β	SC1	SC2	SC3	SC4	SC5
SC1	-0.013	0.012				
SC2	0.089	-0.051	-0.134			
SC3	0.027	0.089	0.001	0.242*		
SC4	-0.098*	-0.128*	0.056	-0.044	-0.006	
SC5	-0.064	0.044	0.088	-0.084	-0.109*	0.000

Randomization tests (10,000 permutations): *p < 0.05

Table S10. The M matrix of eigenvectors for the canonical analysis of γ when males were in the defensive role of first male

	M					Selection	
	SC1	SC2	SC3	SC4	SC5	θ_i	λ_i
m_1	0.376	-0.099	0.877	-0.253	-0.126	0.043	0.152**
m_2	0.459	-0.015	-0.285	-0.596	0.594	0.006	0.090**
m_3	-0.349	0.622	0.345	0.141	0.593	0.017	-0.004
m_4	0.720	0.277	-0.091	0.628	0.037	-0.051	-0.064**
m_5	0.085	0.725	-0.148	-0.408	-0.528	0.133*	-0.118*

The linear (θ_i) and quadratic (λ_i) gradients of selection along each eigenvector are given in the last two columns. Randomization tests (10,000 permutations): *p < 0.05, **p < 0.01

Table S11. First principal components from separate analyses of the measurements of 5 genital sclerites (SC1-SC5) of male *Onthophagus taurus* mating in the offensive role of second male in sperm competition trials

Trait	SC1	SC2	SC3	SC4	SC5
Eigenvalue	1.061	1.856	2.057	2.466	2.092
% variance	53.05	61.86	68.36	82.22	52.31
A-B	0.728	0.635	0.623		0.369
A-C					0.758
B-D				0.865	
area		0.831	0.885	0.923	0.887
outline	0.728	0.873	0.938	0.930	0.772

Table S12. Standardized linear (β), and matrix of quadratic and correlational selection gradients (γ) acting on the five genital sclerites of male *Onthophagus taurus* mating in the offensive role of first male

		γ				
	β	SC1	SC2	SC3	SC4	SC5
SC1	-0.011	0.026				
SC2	0.026	-0.032	-0.026			
SC3	0.054*	-0.009	0.005	-0.018		
SC4	-0.009	0.004	-0.010	0.019	0.016	
SC5	-0.027	0.032*	0.017	-0.008	-0.015	-0.056*

Randomization tests (10,000 permutations): *p < 0.05

Table S13. M matrix of eigenvectors for the canonical analysis of γ when males were in the offensive role of second male

	M					Selection	
	SC1	SC2	SC3	SC4	SC5	θ_i	λ_i
m_1	0.894	-0.345	-0.172	0.010	0.230	-0.034	0.025*
m_2	0.034	-0.211	0.358	0.883	-0.217	0.012	0.015
m_3	0.252	0.614	0.626	-0.016	0.410	0.036*	-0.012*
m_4	0.009	-0.510	0.669	-0.462	-0.280	0.034	-0.016
m_5	-0.370	-0.446	0.060	0.082	0.809	-0.026	-0.041**

The linear (θ_i) and quadratic (l_i) gradients of selection along each eigenvector are given in the last two columns. Randomization tests (10,000 permutations): *p < 0.05, **p < 0.01

Table S14. Least squares allometric scaling relationships between log genital traits and log pronotum width

trait	allometric slope (±SE)
Aedeagus measure	
A-B	0.24±0.09***
B-C	0.29±0.05***
C-G	0.29±0.08***
D-E	0.17±0.04***
E-F	0.26±0.07***
F-G	0.32±0.10**
G-D	0.24±0.10**
G-A	0.13±0.08
Sclerite (measure)	
1 (A-B)	0.27±0.09***
2 (A-B)	0.18±0.04***
2 (B-C)	0.16±0.03***
2 (A-C)	0.25±0.08**
3 (A-B)	0.15±0.04***
3 (B-C)	0.16±0.05**
3 (C-A)	0.23±0.16
4 (A-C)	0.19±0.08*
4 (B-D)	0.35±0.06***
5 (A-B)	0.22±0.04***
5 (A-C)	0.32±0.07***
5 (A-D)	0.26±0.07***
5 (A-E)	0.40±0.30

**p<0.01; ***p<0.001

Table S15. ANCOVA examining homogeneity of slopes between aedeagus size (PC1) and body size for male *Onthophagus taurus* from 3 replicate lines evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Trait Source	SS	df	MS	F	P
Selection history	2.62	1	2.62	0.31	0.609
Line[selection history]	34.60	4	8.65	3.25	0.146
Pronotum	75.01	1	75.01	33.20	<0.001
Pronotum × Selection	1.30	1	1.30	0.49	0.519
Pronotum × Line[selection history]	10.59	4	2.65	1.17	0.325
Error	404.45	179			

A significant interaction term between selection history and pronotum width would indicate different scaling relationships between enforced monogamy and sexual selection lines. SS, sums of squares; df, degrees of freedom; MS, mean squares; F, variance ratio; P, probability

Table S16. ANCOVA examining homogeneity of slopes between genital sclerite size (PC1) and standardized body size for male *Onthophagus taurus* from 3 replicate lines evolving under sexual selection and 3 replicate lines evolving under enforced monogamy

Trait Source	SS	df	MS	F	P
Sclerite 1[1]					
Selection history	0.05	1	0.05	1.40	0.301
Line[selection history]	0.15	4	0.04	0.85	0.556
Pronotum	0.80	1	0.80	7.78	0.006
Pronotum × Selection	0.02	1	0.02	0.33	0.589
Pronotum × Line[selection history]	0.17	4	0.04	0.42	0.795
Error	16.14	156			
Sclerite 2					
Selection history	7.36	1	7.36	3.08	0.154
Line[selection history]	9.57	4	2.39	5.11	0.053
Pronotum	33.14	1	33.14	28.59	<0.001
Pronotum × Selection	1.83	1	1.83	3.71	0.104
Pronotum × Line[selection history]	1.76	4	0.44	0.38	0.823
Error	171.53	148			
Sclerite 3					
Selection history	0.23	1	0.23	0.67	0.452
Line[selection history]	18.47	4	4.62	13.53	0.008
Pronotum	14.51	1	14.51	9.89	0.002
Pronotum × Selection	0.39	1	0.39	0.96	0.357
Pronotum × Line[selection history]	1.27	4	0.32	0.21	0.929
Error	224.45	153			
Sclerite 4					
Selection history	0.00	1	0.00	0.00	0.995
Line[selection history]	13.99	4	3.50	4.71	0.077
Pronotum	4.82	1	4.82	4.15	0.043
Pronotum × Selection	1.24	1	1.24	1.60	0.260
Pronotum × Line[selection history]	2.95	4	0.74	0.63	0.639
Error	181.37	156			
Sclerite 5					
Selection history	0.09	1	0.09	0.02	0.883
Line[selection history]	14.59	4	3.65	1.15	0.446
Pronotum	33.99	1	33.99	17.05	<0.001
Pronotum × Selection	0.13	1	0.13	0.04	0.848
Pronotum × Line[selection history]	12.71	4	3.18	1.59	0.179
Error	291.05	146			

Significant interaction terms between selection history and pronotum width would indicate different scaling relationships between enforced monogamy and sexual selection lines.

[1]Sums of squares and mean squares $\times 10^{-2}$