

Electronic supplementary material

Adaptive shaping of the behavioural and neuroendocrine phenotype during adolescence

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Material and methods

Experimental design and procedures

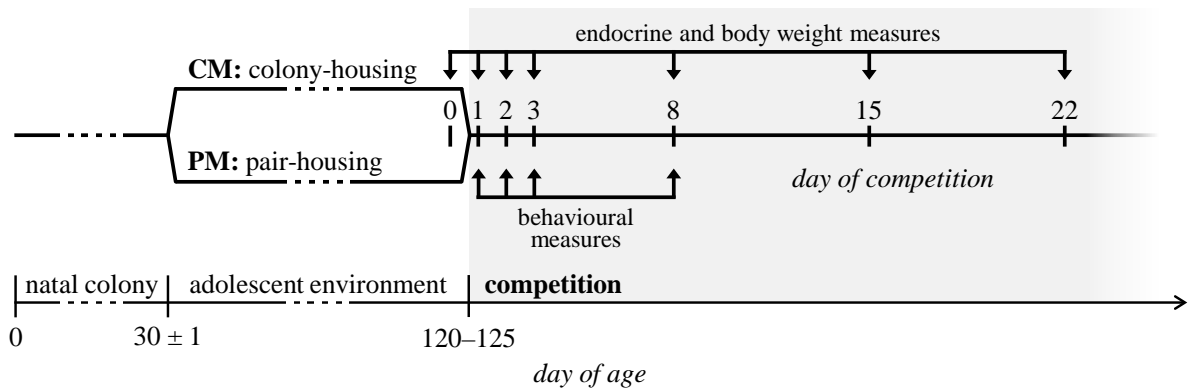


Figure S1. Experimental design. Colony-born males were transferred to different adolescent social environments at 30 (± 1) days of age and subsequently housed either in another colony (CM) or pairwise with one female (PM). At 120–125 days of age (i.e. in late adolescence), highly competitive situations with a single male for mating access were created by establishing groups of one pair-housed male, one colony-housed male and two females ($n = 13$). Plasma cortisol and testosterone concentrations and body weights were determined 20 h before competition (day 0), 4 h after the onset of competition on day 1, and then again on days 2, 3, 8, 15 and 22. Behaviour was recorded the first 3 h of competition on day 1 and for 2 h each on days 2, 3 and 8.

Behavioural measures

Table S2. Behavioural categories and definitions.

Agonistic behaviour	
<i>Mild agonistic behaviour</i>	
Retreat	Subject moves to at least one body length from opponent following agonistic interaction or approach by opponent.
<i>Threat display</i>	
Head up	Subject throws head back with nose pointing upwards.
Curved body posture	Subject stands sideways in front of opponent, body is curved with head and rump directed to opponent.
<i>Escalated aggression</i>	
Attack lunge	Subject darts at opponent.
Chase	Subject runs after opponent.
Head thrust	Subject shoves opponent with its snout.
Turn around	Two animals fight in direct contact and try to turn opponent onto its back.
Sociopositive behaviour	
Nose contact	Subject touches another animal with its nose.
Courtship & sexual behaviour	
Anogenital licking	Subject repeatedly sniffs and/or licks female's anogenital region.
Chin-rump follow	Subject presses head on female's rump and follows her.
Mount	Subject places front body on female's dorsal surface.
Rumba	Subject rhythmically oscillates its hindquarters from side to side and emits a burbling vocalization (rumble). Head stretches forward parallel to the ground and subject's body may assume a curved posture as it nears female.
Rumping	Subject throws one or both hind legs over female's back or rump.
Other behaviour	
Approach	Subject moves to within one body length of another animal.

Hormones

Table S3. Antibody cross-reactivity of testosterone enzyme-linked immunosorbent assay.

Substance	Cross reactivity
Androstenedione	32.2%
5 α -Dihydrotestosterone	23.3%
Androsterone	< 0.1%
5 α -Androstane	< 0.1%
5 β -Androstane-3 α ,17 β -diol	< 0.1%
Corticosterone	< 0.1%
11-Desoxycorticosterone	< 0.1%
Dexamethasone	< 0.1%
Estradiol	< 0.1%
Progesterone	< 0.1%
17 α -Hydroxyprogesterone	< 0.1%
Cortisol	< 0.1%
11-Desoxycortisol	< 0.1%
Cortisone	< 0.1%
Estrone	< 0.1%
Pregnenolone	< 0.1%
Prednisone	< 0.1%
Prednisolone	< 0.1%
Danazol	< 0.1%

Table S4. Antibody cross-reactivity of cortisol luminescence immunosorbent assay.

Substance	Cross reactivity
Prednisolone	29.8%
11-Desoxycortisol	8.48%
Cortisone	4.49%
Prednisone	2.12%
Corticosterone	1.99%
6 β -Hydroxycortisol	1.03%

Paternity assignment

Allele frequency analysis included all typed individuals ($n = 94$). Since maternities were ambiguous in some cases, offspring were assigned to parent pairs with known sexes involving two candidate fathers and either the known mother or two candidate mothers each. All candidate parents were sampled with a minimum number of five typed loci. The proportion of genotyping errors was estimated by checking known independent mother-offspring pairs ($n = 13$) for allele mismatches (cf. Hoffman & Amos 2005), yielding a mean error rate across loci of 0.0078. Parentages were assigned based on simulations of 100,000 cycles.

Data analysis

Table S5. Benjamini-Hochberg corrected significance levels for multiple comparisons of repeated measures.

Measure	Corrected significance level
<i>Behavioural measures</i>	
Approaching opponent	0.038
Retreat	0.038
Escalated aggression	0.025
Dominance	0.038
Approaching females	0.05
Courtship and sexual behaviour	0.025
Sociopositive behaviour	0.025
Approach by females	0.038
<i>Endocrine and body weight measures</i>	
Plasma testosterone concentration	
Relative	0.042
Absolute	0.014
Plasma cortisol concentration	
Relative	0.008
Absolute	0.007
Body weight	0.008

Results

Behavioural measures

Table S6. Median (first to third quartiles) latencies (in s) to the first occurrence of each behavioural measure. Latencies were set to the maximum total observation time of 9 h (= 32,400 s) if a subject did not exhibit the respective behaviour at all. PMs = pair-housed males, CMs = colony-housed males. Statistics: Wilcoxon signed-rank test (two-tailed). Significant differences are indicated by bold numbers.

Behavioural measure	Latency (in s)		Statistics		
	PMs	CMs	W	n	p
<i>Male-male interactions</i>					
Approaching opponent	67 (45–195)	150 (97–197)	32	13	0.376
Threat display	472 (158–2,031)	829 (181–18,553)	27	10	0.738
Escalated aggression	1,008 (500–2,960)	1,439 (112–32,400)	20	12	0.151
Retreat	764 (222–32,400)	354 (219–739)	20	11	0.278
<i>Male-female interactions</i>					
Approaching female	34 (7–115)	7 (3–29)	25.5	13	0.173
Sociopositive behaviour	934 (257–2,365)	1,691 (847–3,200)	30	13	0.305
Courtship and sexual behaviour	264 (154–398)	563 (130–8,069)	10	11	0.042
Approach by female	63 (47–179)	176 (132–400)	29	13	0.273

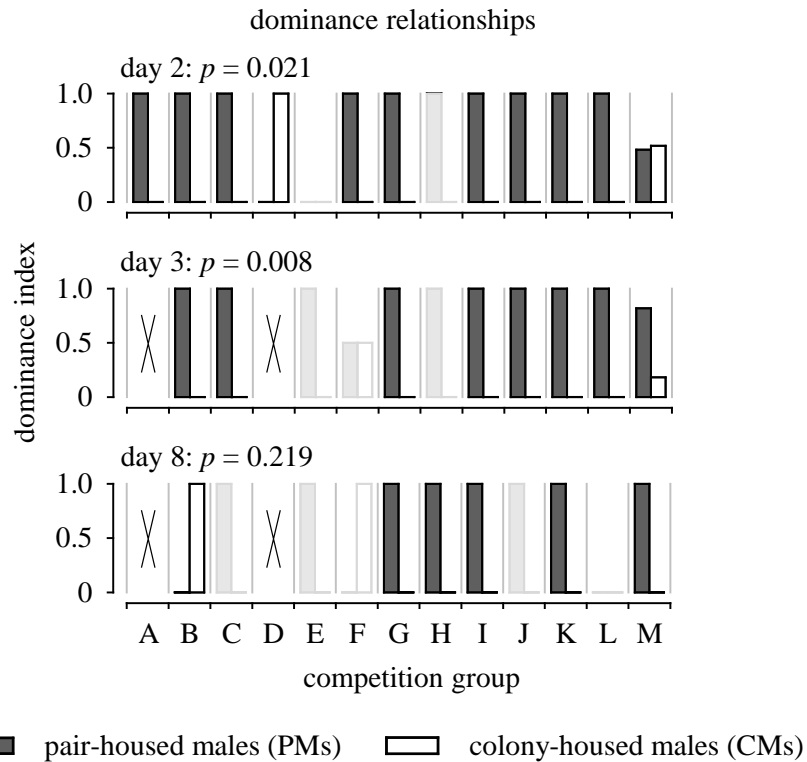


Figure S7. Dominance indices on days 2, 3 and 8 after onset of competition for each group (A–M). Index is based on outcomes of agonistic encounters and ranges from 0 (absolutely subordinate) to 1 (absolutely dominant). Faded coloured columns: insufficient data to calculate index; crossed columns: terminated group. Statistics: Wilcoxon signed-rank test (two-tailed) with Benjamini-Hochberg correction (corrected significance level = 0.038).

Endocrine and body weight measures

Table S8. Median (first to third quartiles) absolute plasma testosterone concentrations (in ng ml^{-1}) on the day before (day 0) and days 1, 2, 3, 8, 15 and 22 after onset of competition. PMs = pair-housed males, CMs = colony-housed males. Statistics: Wilcoxon signed-rank test (two-tailed) with Benjamini-Hochberg correction (corrected significance level = 0.014). Statistically significant differences are indicated by bold numbers.

Day	Plasma testosterone (ng ml^{-1})		Statistics		
	PMs	CMs	W	<i>n</i>	<i>p</i>
0	2.4 (1.5–3.1)	6.3 (4.1–8.2)	8	13	0.006
1	5.8 (4.9–6.9)	5.7 (2.8–7.6)	24	12	0.254
2	5.1 (3.8–5.8)	2.8 (1.9–4.0)	4	11	0.007
3	4.8 (3.4–6.4)	2.6 (2.2–3.5)	10	11	0.042
8	2.4 (1.5–2.6)	2.5 (1.9–4.8)	15	11	0.123
15	2.4 (1.8–2.7)	2.3 (1.7–3.0)	16	7	0.813
22	2.3 (1.8–3.2)	2.9 (1.9–5.9)	6	8	0.109

Table S9. Median (first to third quartiles) absolute plasma cortisol concentrations (in ng ml⁻¹) on the day before (day 0) and days 1, 2, 3, 8, 15 and 22 after onset of competition. PMs = pair-housed males, CMs = colony-housed males. Statistics: Wilcoxon signed-rank test (two-tailed) with Benjamini-Hochberg correction (corrected significance level = 0.007).

Day	Plasma cortisol (ng ml ⁻¹)		Statistics		
	PMs	CMs	W	n	p
0	229 (171–271)	290 (238–373)	37.5	13	0.6
1	521 (451–629)	456 (304–554)	19	13	0.068
2	445 (312–525)	476 (423–568)	36	12	0.85
3	365 (298–699)	354 (307–382)	18.5	11	0.215
8	299 (254–471)	282 (179–350)	1.5	9	0.012
15	337 (292–511)	211 (177–254)	2	6	0.094
22	316 (272–509)	248 (213–289)	5	8	0.078

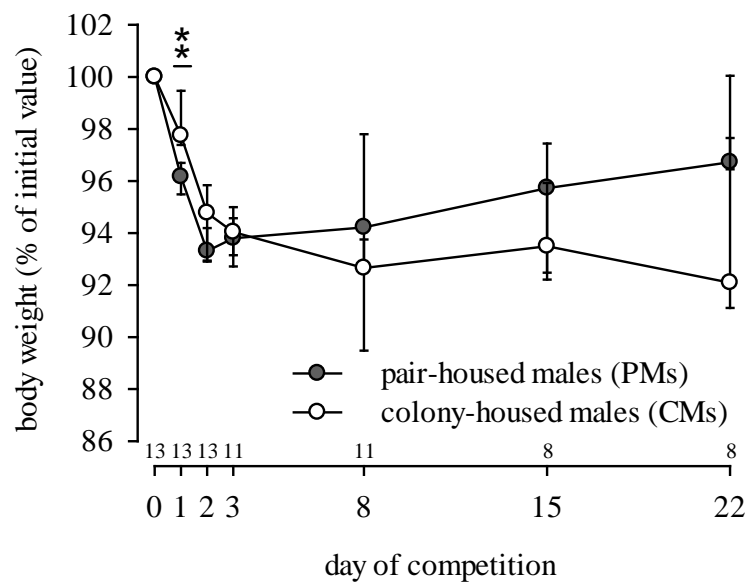


Figure S10. Median (first to third quartiles) body weights on days 1, 2, 3, 8, 15 and 22 after onset of competition. Values are presented in per cent of baseline values determined 20 h before competition (day 0). Numbers above the x-axis denote sample size. Statistics: Changes over time: Skillings-Mack test: d.f. = 6, n = 13; PMs: $p < 0.001$, CMs: $p < 0.001$. Pairwise comparisons: Wilcoxon signed-rank test (two-tailed) with Benjamini-Hochberg correction (corrected significance level = 0.008): ** $p = 0.002$.

Supplementary References

Hoffman JI, Amos W. 2005 Microsatellite genotyping errors: detection approaches, common sources and consequences for paternal exclusion. *Mol. Ecol.* **14**, 599–612.
(doi:10.1111/j.1365-294X.2004.02419.x)