Supplementary materials

Are behaviour and stress-related phenotypes in urban birds adaptive?

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FIGURES

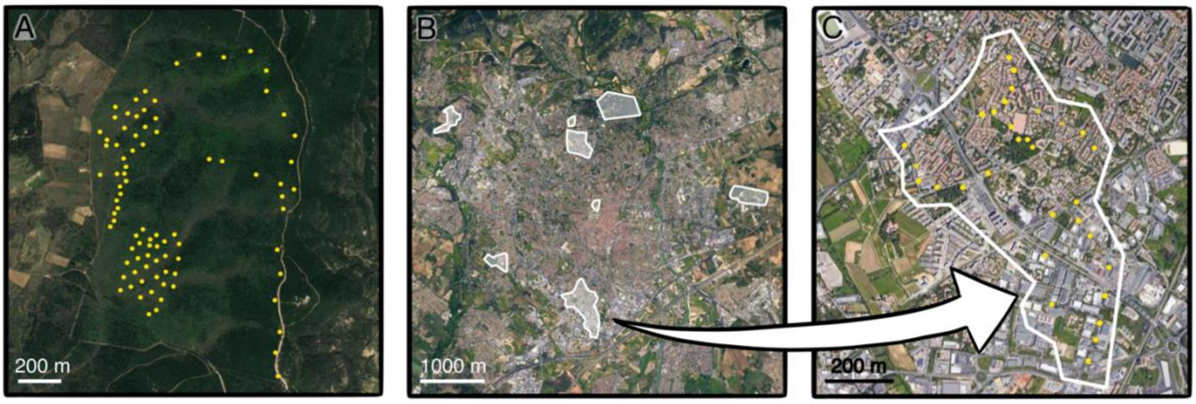

Figure S1: Satellite images of the study sites**.** (A) The forest of La Rouvière near Montarnaud, France. (B) Map of Montpellier showing the eight monitored areas of the city in white. (C) Zoom of a section of one urban site. Yellow dots represent great tit nest-boxes (entrance whole of 32 mm). Note that the forest site is also equipped with blue tit nest-boxes (entrance of 28 mm) which are not shown on this map. ©Google Earth.

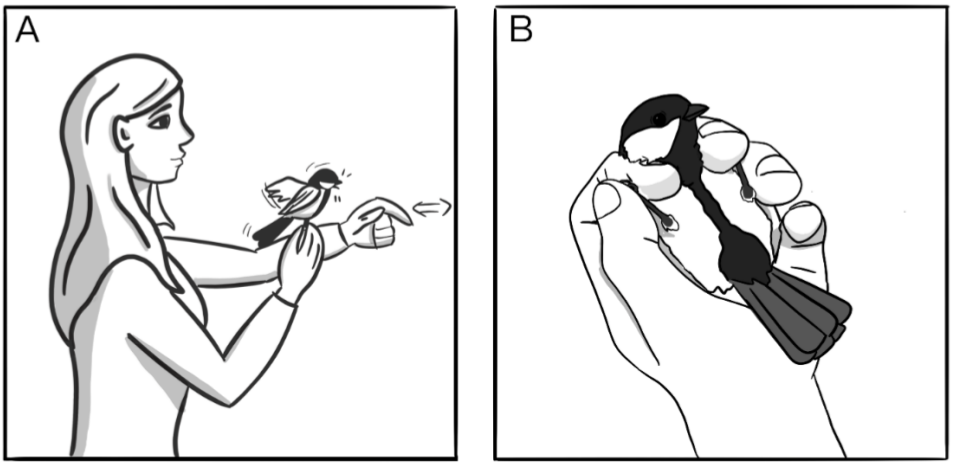

Figure S2: (A) Position of handler for the handling aggression (HA) test. During the test, the bird's legs are held still by one hand and the bird is nagged with a finger of the other hand. (B) Bird held on its back to perform the measure of Breath Rate Index (BRI).

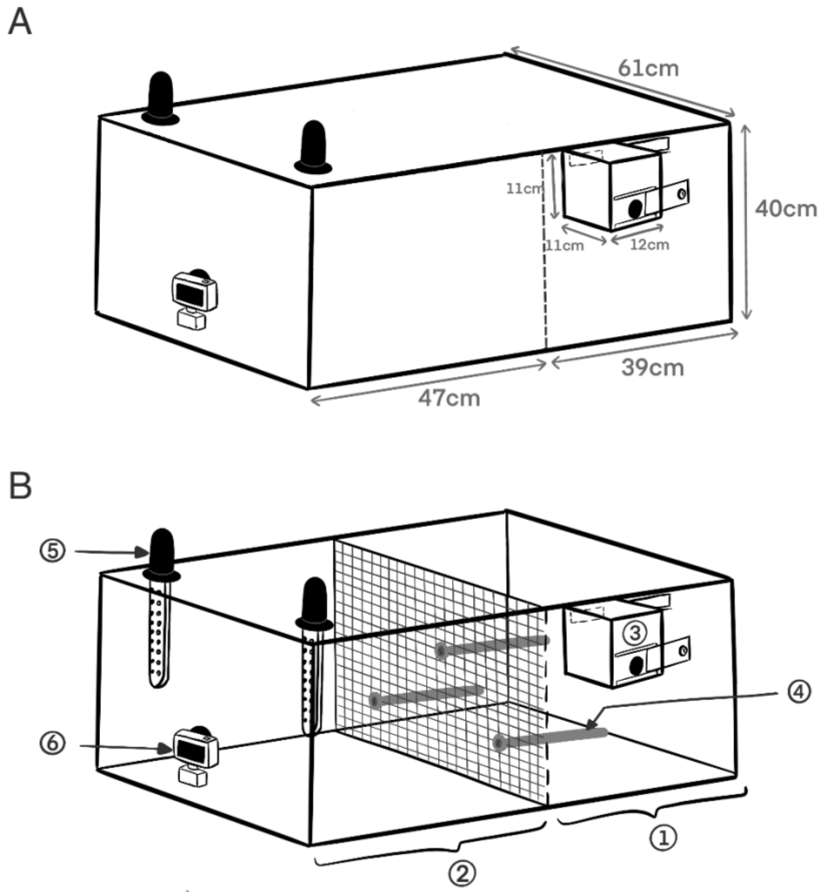

Figure S3: Drawing of the experimental open-field cage. (A) Outside view and detailed dimensions (B) Transparent view of the internal structure of the cage. 1: exploration room with three perches, 2: empty area inaccessible for the bird, 3: acclimatization compartment, 4: perch, 5: light and 6: camera. A sliding door connects the acclimatization compartment to the experimental area. External walls are made of expanded PVC sheets.

TABLES

Table S1: Bird handling aggression scale**.** See Figure S2A for an illustration of how the bird should be handled. Note that when a bird had one reaction specific to a score and another reaction specific to another score, the attributed score was an average of the two.

Score	Bird strickes handler	Tail spread	Wings spread
0	No	No	No
1	Yes, but only if provoked	No	No
2	Yes, spontaneously	Yes	No
3	Yes, spontaneously	Yes	Yes

Table S2: Model selection for the recapture probability (p) from the complete model including habitat, sex, time (*i.e.* year) and handling aggression (HA) or breath rate index (BRI) or exploration score (ES) of breeding great tits**.** Models are ranked according to QAICc values, and only the five best models (i.e. lowest QAICc) are presented for each trait-specific dataset. Supported models (ΔQAICc<2) are represented in bold.

Models	# Par.	Deviance	QAICc	ΔQAICc
Handling aggression				
$p_.$	**5**	**1437.46**	**1173.98**	**0**
p_{hab}	**6**	**1436.07**	**1174.89**	**0.90**
p_{sex}	**6**	**1437.40**	**1175.96**	**1.97**
$p_{hab+sex}$	7	1436.01	1176.86	2.88
p_{hab+HA}	7	1436.03	1176.88	2.89
Breath Rate index				
$p_.$	**5**	**1272.61**	**897.52**	**0**
$p_{hab+BRI}$	**7**	**1266.98**	**897.63**	**0.12**
p_{hab}	**6**	**1271.47**	**898.74**	**1.23**
$p_{sex+BRI}$	**7**	**1269.41**	**899.34**	**1.82**
p_{sex}	**6**	**1272.54**	**899.49**	**1.97**
Exploration Score				
$p_.$	**5**	**1264.91**	**875.78**	**0**
p_{hab}	**6**	**1263.81**	**877.05**	**1.27**
p_{sex}	**6**	**1264.87**	**877.77**	**1.99**
p_{hab+ES}	7	1263.75	879.03	3.26
$p_{hab+sex}$	7	1263.76	879.04	3.26

Table S3: Results of the goodness-of-fit tests performed on the overall dataset with 4 groups of individuals (forest females, forest males, urban females and urban males). Data showed a good fit to the assumptions of the general CJS model.

Test	Chi-square	df	P
Forest females			
Test 3.SR	8.755	5	0.119
Test 3.SM	0.443	2	0.801
Test 2.CT	$4x10^{-30}$	1	1
Forest males			
Test 3.SR	12.653	5	0.025
Test 3.SM	0.936	3	0.817
Test 2.CT	0.559	3	0.906
Urban females			
Test 3.SR	5.711	5	0.335
Test 3.SM	5.789	3	0.122
Test 2.CT	4.783	4	0.310
Urban males			
Test 3.SR	9.256	5	0.099
Test 3.SM	0.762	3	0.858
Test 2.CT	7.048	4	0.133

Table S4: Between-individual (V_I) and residual (V_R) variance components and their upper and lower credibility intervals for the behavioural and physiological traits, estimates are extracted from the posterior modes of a bivariate mixed model, using a Bayesian framework (MCMCglmm procedure).

Habitat	Trait	DIC	V_I	Lower	Upper	V_R	Lower	Upper
Urban	Exploration score	3530.349	8.566	5.797	11.41	9.652	7.488	11.940
	Breath rate index	1708.495	0.510	0.362	0.661	0.541	0.431	0.664
	Handling aggression	2170.638	0.126	0.070	0.189	1.385	1.227	1.546
Forest	Exploration score	1034.203	12.92	8.362	17.65	4.759	2.498	7.378
	Breath rate index	782.860	0.359	0.122	0.651	2.171	1.683	2.695
	Handling aggression	592.774	0.299	0.127	0.492	0.877	0.632	1.115

<u>Table S5</u>: Individual ($Cov_{I1,2}$) and residual ($Cov_{R1,2}$) covariances between behavioural and physiological traits and their lower and upper credibility intervals, estimates are extracted from the posterior modes of a bivariate mixed model, using a Bayesian framework (MCMCglmm procedure).

Habitat	Trait 1	Trait 2	Model*	DIC	$Cov_{I1,2}$	Lower	Upper	$Cov_{R1,2}$	Lower	Upper
Urban	ES	HA	1	5356.368	-0.675	-1.538	0.163	0.052	-0.325	0.441
			2	**5356.389**	**-**	**-**	**-**	**-0.065**	**-0.425**	**0.283**
	ES	BRI	1	5826.585	-1.515	-2.773	-0.337	-0.144	-0.622	0.354
			2	**5819.144**	**-**	**-**	**-**	**-0.222**	**-0.692**	**0.269**
	HA	BRI	1	4145.169	-0.539	-0.728	-0.351	-0.277	-0.424	-0.138
			2	**4236.452**	**-**	**-**	**-**	**-0.572**	**-0.725**	**-0.422**
Forest	ES	HA	1	1631.786	0.266	-0.713	1.277	0.059	-0.532	0.649
			2	**1632.408**	**-**	**-**	**-**	**0.125**	**-0.401**	**0.624**
	ES	BRI	1	**1802.463**	**-2.025**	**-3.797**	**-0.297**	**-0.088**	**-0.952**	**0.706**
			2	1806.048	-	-	-	-0.437	-1.295	0.371
	HA	BRI	1	**1459.508**	**-0.283**	**-0.638**	**0.059**	**-0.291**	**-0.686**	**0.091**
			2	1469.911	-	-	-	-0.486	-0.830	-0.153

* model 1 : estimating all covariances, model 2 : fixed individual covariance

<u>Table S6:</u> Contrasting Great tit handling aggression, breath rate index and exploration score across habitat, year, sex, age, temperature and rank of capture (and mass and hour of the day for breath rate index). Estimates are calculated by model averaging of equally supported best models presented in Table 3. The upper section of the table indicates estimates for fixed effects. The lower section of the table presents variance components and sample sizes.

	Handling Aggression				Breath Rate Index				Exploration Score			
Fixed effects	**Est.**	**SE**	**z**	**P**	**Est.**	**SE**	**z**	**P**	**Est.**	**SE**	**z**	**P**
Intercept	1.775	0.172	10.325	**< 2x10^{-16}**	0.271	0.921	0.294	0.769	3.775	0.857	4.4	**1.1x10^{-5}**
Habitat-Urban	0.4194	0.108	3.937	**8.3x10^{-5}**	-0.453	0.099	4.577	**4.7x10^{-6}**	2.494	0.472	5.282	**1.0x10^{-7}**
Year-2015	-0.143	0.105	1.356	0.175	0.279	0.150	1.863	0.062	n.s.	n.s.	n.s.	n.s.
Year-2016	-0.235	0.108	2.168	**0.03**	0.369	0.153	2.406	**0.016**	n.s.	n.s.	n.s.	n.s.
Year-2017	0.071	0.114	0.324	0.533	0.781	0.196	3.981	**6.9x10^{-5}**	n.s.	n.s.	n.s.	n.s.
Year-2018	-0.07	0.114	0.615	0.539	0.767	0.172	4.445	**8.8x10^{-6}**	n.s.	n.s.	n.s.	n.s.
Year-2019	-0.115	0.113	1.011	0.312	0.619	0.163	3.785	1.5x10^{-4}	n.s.	n.s.	n.s.	n.s.
Sex-Female	-0.068	0.117	0.58	0.562	-0.082	0.089	0.920	0.358	n.s.	n.s.	n.s.	n.s.
Age-Yearling	n.s.	n.s.	n.s.	n.s.	-0.027	0.096	0.280	0.779	-0.023	0.123	0.185	0.854
Capture rank	n.s.	n.s.	n.s.	n.s.	-0.168	0.057	2.993	**0.003**	-0.228	0.209	1.087	0.277
Temperature	0.001	0.006	0.143	0.886	0.048	0.010	4.761	**1.9x10^{-6}**	0.03	0.034	0.887	0.375
Mass	-	-	-	-	-0.081	0.046	1.762	**0.078**	-	-	-	-
Hour of day	-	-	-	-	n.s.	n.s.	n.s.	n.s.	-	-	-	-
Hab-Urb × Sex-F	-0.464	0.141	3.292	**9.9x10^{-4}**	n.s.	n.s.	n.s.	n.s.	0.012	0.435	0.028	0.977
Random effects	**Variance**		**Sample size**		**Variance**		**Sample size**		**Variance**		**Sample size**	
Individual ID	0.445		nb. Indiv=812		0.138		nb indiv=675		9.42		nd indiv=667	
Manipulator ID	0.036		nb. manip=22		2.3x10^{-9}		nb manip=7		-		-	
Residuals	0.567		nb. Obs = 1302		1.414		nb obs=874		8.56		nb obs=850	

Table S7: Model selection for models estimating reproductive linear selection gradients for handling aggression (HA), breath rate index (BRI) and exploration score (ES). Models in bold are equally supported (ΔQAICc<2). Estimates obtained by model averaging of the best models selected here are available in main text Table 6.

Models	# Par.	Deviance	AICc	ΔAICc
Females				
HA + BRI + ES + habitat + habitat*HA + habitat*ES	**10**	**1005.60**	**1026.20**	**0**
HA + BRI + ES + habitat + habitat*HA	**9**	**1008.06**	**1026.55**	**0.36**
HA + BRI + ES + habitat	**8**	**1043.48**	**1026.88**	**0.69**
HA + BRI + ES + habitat + habitat*ES	**9**	**1008.64**	**1027.13**	**0.93**
HA + BRI + ES + habitat + habitat*HA + habitat*BRI + habitat*ES	11	1005.56	1028.28	2.08
HA + BRI + ES + habitat + habitat*HA + habitat*BRI	10	1008.05	1028.65	2.45
HA + BRI + ES + habitat + habitat*BRI	9	1010.49	1028.98	2.79
HA + BRI + ES + habitat + habitat*BRI + habitat*ES	10	1008.63	1029.23	3.03
Males				
HA + BRI + ES + habitat + habitat*ES	**9**	**900.11**	**918.65**	**0**
HA + BRI + ES + habitat + habitat*BRI + habitat*ES	**10**	**899.98**	**920.65**	**2.00**
HA + BRI + ES + habitat + habitat*HA + habitat*ES	10	900.08	920.75	2.10
HA + BRI + ES + habitat	8	905.1234	921.56	2.91
HA + BRI + ES + habitat + habitat*HA + habitat*BRI +habitat*ES	11	899.97	922.78	4.13
HA + BRI + ES + habitat + habitat*BRI	9	904.94	923.49	4.84
HA + BRI + ES + habitat + habitat*HA	9	905.12	923.67	5.02
HA + BRI + ES + habitat + habitat*HA + habitat*BRI	10	904.94	925.60	6.95

Table S8: Model selection for models estimating reproductive quadratic selection differentials for handling aggression (HA), breath rate index (BRI) and exploration score (ES) following equation (S4) Appendix 3.

Models	# Par.	Deviance	AICc	ΔAICc
Hangling agression				
Females				
HA + habitat + HA* habitat + HA^2	8	1046.86	1063.236	0
HA + habitat + HA* habitat + HA^2 +HA^2*habitat	9	1046.38	1064.852	1.62
Males				
HA + habitat + HA* habitat + HA^2	8	945.31	961.7273	0
HA + habitat + HA* habitat + HA^2 +HA^2*habitat	9	944.41	962.9361	1.21
Breath rate index				
Females				
BRI + habitat + BRI* habitat + BRI^2	8	1070.72	1087.09	0
BRI + habitat + BRI* habitat + BRI^2 + BRI^2*habitat	9	1069.96	1088.42	1.33
Males				
BRI + habitat + BRI* habitat + BRI^2	8	984.46	1000.86	0
BRI + habitat + BRI* habitat + BRI^2 + BRI^2*habitat	9	983.09	1001.60	0.74
Exploration score				
Females				
ES + habitat + ES* habitat + ES^2	8	1709.81	1726.04	0
ES + habitat + ES* habitat + ES^2 +ES^2*habitat	9	1709.48	1727.78	1.73
Males				
ES + habitat + ES* habitat + ES^2	8	1564.95	1581.21	0
ES + habitat + ES* habitat + ES^2 +ES^2*habitat	9	1564.51	1582.84	1.63

<u>Table S9:</u> Model selection for models estimating reproductive quadratic selection gradients and correlational selection for handling aggression (HA), breath rate index (BRI) and exploration score (ES) following equation (S5). Models in bold are equally supported (ΔQAICc<2). Estimates obtained by model averaging of the best models selected here are available in main text Table 7.

Models	# Par.	Deviance	AICc	ΔAICc
Females				
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES	**14**	**999.08**	**1028.24**	**0**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + ES^2*habitat	**15**	**998.01**	**1029.33**	**1.09**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat	**15**	**998.88**	**1030.21**	**1.97**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat	15	999.05	1030.37	2.13
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat + ES^2*habitat	16	997.83	1031.33	3.10
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + ES^2*habitat	16	997.90	1031.41	3.17
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat	16	998.84	1032.35	4.11
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat + ES^2*habitat	17	997.72	1033.41	5.18
Males				
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES	**14**	**897.16**	**926.45**	**0**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat	15	897.02	928.50	2.05
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + ES^2*habitat	15	897.10	928.59	2.13
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat	15	897.11	928.59	2.14
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat + ES^2*habitat	16	896.98	930.67	4.21
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat	16	896.99	930.68	4.22
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + ES^2*habitat	16	897.05	930.73	4.28
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat + ES^2*habitat	17	896.95	932.85	6.40

APPENDIX

Appendix 1: Impact of change of protocol on Breath Rate Index (BRI)

For the period of 2013 to 2016 breath rate was estimated using the **number of chest movements during 30s (method 1)**. In 2017, the protocol was updated to measure the **time to complete 30 chest movements (method 2)**, which was easier to perform. Measures from 2013 to 2016 were converted to approximate the time needed to complete 30 chest movements to obtain similarly scaled measures as the 2017 measure. In order to test whether the shift in the method had a significant impact on the measure of breath rate, we explored potential differences in variances and means associated with the method. Measures of breath rate were significantly lower with method 2 ($mean_{method1}$ = 14.182, $mean_{method2}$ = 12.998, Wilcoxon test: W = 66902, P = 1.95×10^{-14}).

The most likely explanation for a lower mean with method 2 is that method 1 of counting the number of chest movements during 30s took longer than method 2, hence birds had time to calm down and reduce their breath rate during this relatively long handling time. Method 1 being quicker, we believe it better captured the immediate response to handling stress.

Based on these results, we performed a linear regression between breath rate measure and method and extracted the residuals. Residuals of this regression are used as breath rate index (BRI) in the article.

<u>Appendix 2:</u> Analyses of correlation between traits

To test whether the behavioural and stress response traits studied here were correlated with each other at the within-individual and between-individual levels, we estimated variance and covariance components using a Bayesian linear mixed model approach (MCMCglmm package in R, Hadfield 2010) in each habitat separately. We chose a Bayesian approach for this analysis because MCMCglmm is one of the only packages running bivariate mixed models (Dingemanse & Dochtermann 2013). Each bivariate mixed model included two traits as dependent variables in order to decompose trait variance and covariances. Models were built following Dingemanse & Dochterman (2013) and Ferrari et al. (2013):

$$x_{1,ij} = \beta_{0x1} + \text{ind}_{0x1,ij} + \beta_1 a_{1x1,ij} + e_{0x1,ij} \qquad \text{(S1)}$$

$$x_{2,ij} = \beta_{0x2} + \text{ind}_{0x2ij} + \beta_1 a_{1x2ij} + e_{0x2,ij} \qquad \text{(S2)}$$

where $x_{1,ijk}$ and $x_{2,ijk}$ are two different traits measured in individual i at time j, β_0 are intercepts, β_1 are coefficients relating fixed effects a to the dependent variables, ind_0 are the random intercepts of the individual deviation from the mean and e_0 are the random error terms. Models included all fixed effect that were found significant in the best models from the analyses of phenotypic divergence (main text Table 3). To run these models, we used only non-informative priors since the use of informative prior is currently debated. We used 300 500 iterations, thinning intervals of 10 and a burn-in of 1 000.

The total phenotypic (co)variances in all three focal traits were decomposed into individual (V_I and $Cov_{I1,2}$) and residual (V_R and $Cov_{R1,2}$) components as follow:

$$\begin{bmatrix}\text{ind}_{0x1j}\\ \text{ind}_{0x2j}\end{bmatrix} = MVN(0, \Omega_I) : \Omega_I = \begin{bmatrix} V_{I0x1} & \text{Cov}_{I0x1,I0x2} \\ \text{Cov}_{I0x1,I0x2} & V_{I0x2}\end{bmatrix}$$

$$\begin{bmatrix}e_{0x1jk}\\ e_{0x2jk}\end{bmatrix} = MVN(0, \Omega_R) : \Omega_R = \begin{bmatrix} V_{R0x1} & \text{Cov}_{R0x1,R0x2} \\ \text{Cov}_{R0x1,R0x2} & V_{R0x2}\end{bmatrix} \qquad \text{(S3)}$$

<u>Appendix 3:</u> Estimating quadratic selection differentials/gradients on behavioural and stress response traits

In addition to estimating linear reproductive selection, we estimated non-linear (*i.e.* quadratic) selection using reproductive success as fitness proxy. While a linear selection differential/gradient provides an estimate of directional selection, a quadratic selection differential/gradient evaluates the existence and strength of stabilising or disruptive selection (Lande & Arnold, 1983). Quadratic selection differentials (γ) and selection gradients (γ_i for each trait x_i) as well as correlational selection gradients (γ_{ij} for traits x_i and x_j) were estimated for each trait x_i following these two types of models, ran on relative individual fitness ω:

$$\omega = \alpha + \beta x + (\gamma/2)x^2 + \varepsilon \qquad \text{(S4)}$$

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + (\gamma_1/2)x_1^2 + (\gamma_2/2)x_2^2 + (\gamma_3/2)x_3^2 + \gamma_{12}x_1x_2 + \gamma_{23}x_2x_3 + \gamma_{13}x_1x_3 + \varepsilon \quad \text{(S5)}$$

In equations S4 and S5, α represents the intercept, β and β_i represent respectively the linear selection differential and the linear selection gradient for each trait x_i, and ε represents the error. While a positive estimate of quadratic selection differential/gradient indicates disruptive selection, a negative estimate is indicative of stabilising selection.

The model selection procedure was performed following the method described in the main text.

Results for quadratic selection differentials

When estimating quadratic selection differentials on each of the three traits separately, model selection revealed that models with a $\text{trait}^2\times\text{habitat}$ interaction were equally supported (within 2 points of AICc) compared to the null model (Table S8). These results suggest that quadratic selection differentials may vary between the two habitats for any of the three studied traits.