**An experimental analysis of the heritability of variation in glucocorticoid concentrations in a wild avian population**

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**Electronic Supplementary Material**

Supplementary Text. Parentage analysis polymerase chain reaction (PCR) methods (supplemental material for section 2.3 in main text).

We used paternity analyses to verify relatedness among brood-mates due to the high rates of EPFs in our population, but not all experimental brood pairs are represented in our data set as we were unable to verify parentage for some nestlings. The social and genetic parents of the nestlings involved in our experiment were identified using a combination of visual observations and molecular tools. Nestlings were only included in our analyses if we had collected either a baseline blood sample taken within 3 minutes of initial disturbance, a stress-induced blood sample taken between 15-18 minutes, or both, and we were able to get blood samples from both social parents. DNA was extracted from nestling and adult blood samples that were stored in lysis buffer using DNeasy Blood & Tissue Extraction kits (Qiagen, Maryland, U.S.A).

Polymerase chain reaction (PCR) was utilized to amplify six previously developed microsatellite loci (Escu6: [1]; Ltr6: [2]; Pocc6: [3]; and Hir11, Hir19, and Hir20: [4]). Reaction conditions for pooled Escu6, Ltr6, Hir20, and Hir11 primers consisted of a 10 µl solution with 50-100 ng DNA, 0.12 mM of each labeled forward primer, 0.12 mM of each reverse primer, 200 M each dNTP, 3.25 mM $MgCl_2$, 1x PCR Buffer, 0.15 units Taq polymerase (New England

Biolabs, Massachusetts, U.S.A.), and were amplified with the following protocol: initial denaturation step of 94°C for 1 minute, followed by 10 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, with an additional 25 cycles starting at 87°C for 30 s instead of 94°C, and completed with a final extension at 72 °C for 3 min. The Pocc6 reaction was modified from the above conditions by using 1.25 mM $MgCl_2$, and modified for the Hir19 reaction with 3 mM $MgCl_2$ and 0.2 mM of each forward and reverse primer. The PCR amplification protocol for Pocc6 and Hir19 was similar to the pooled loci protocol with the exception that 60°C was used for the annealing temperature. Amplified PCR products containing the fluorescently-labeled forward primer were detected using an ABI3730 DNA analyzer (ABI, Inc.).

## Supplementary Statistical Data

Table S1. Summary of models with different specified priors. The final model included in the main text is in bold.

| | DIC | Baseline CORT | | Stress-Induced CORT | | Genetic Correlation (95% BCI) |
|---|---|---|---|---|---|---|
| | | $h^2$ (95% BCI) | $e^2$ 95% BCI) | $h^2$ (95% BCI) | $e^2$ (95% BCI) | |
| **$V_P$ split evenly** | **1039.088** | **0.152 (0.062 - 0.458)** | **0.555 (0.309 - 0.771)** | **0.343 (0.128 - 0.598)** | **0.491 (0.251 - 0.693)** | **-0.297 (-0.688 - 0.333)** |
| $V_A + V_E = 95\% V_P$; $V_R = 5\% V_P$ | 791.642 | 0.222 (0.069 - 0.6) | 0.59 (0.311 - 0.792) | 0.428 (0.217 - 0.73) | 0.432 (0.235 - 0.69) | -0.244 (-0.706 - 0.309) |
| $V_A + V_E = 90\% V_P$; $V_R = 10\% V_P$ | 881.887 | 0.176 (0.065 - 0.548) | 0.557 (0.321 - 0.794) | 0.408 (0.192 - 0.691) | 0.467 (0.242 - 0.698) | -0.139 (-0.759 - 0.26) |
| $V_A + V_E = 75\% V_P$; $V_R = 25\% V_P$ | 978.857 | 0.151 (0.045 - 0.486) | 0.628 (0.31 - 0.782) | 0.396 (0.15 - 0.658) | 0.464 (0.227 - 0.694) | -0.275 (-0.789 - 0.297) |
| $V_A + V_E = 50\% V_P$; $V_R = 50\% V_P$ | 1038.222 | 0.154 (0.031 - 0.431) | 0.62 (0.297 - 0.774) | 0.278 (0.088 - 0.658) | 0.506 (0.228 - 0.703) | 0.239 (0.101 - 0.467) |

Table S2. Posterior mode (and 95% BCI) of all fixed effects included in maximal model. Fixed effects with a statistical effect (posterior distribution does not overlap zero) that were included in the final model are in bold.

| | Baseline CORT | Stress-Induced CORT |
|---|---|---|
| **Latency Time** | **1.749 (0.949 – 2.529)** | 0.545 (-1.904 – 2.105) |
| **Body Mass** | **-0.690 (-0.972 – -0.380)** | -0.316 (-1.038 – 0.791) |
| Brood Size | 0.145 (-0.510 – 0.899) | -0.204 (-2.411 – 1.624) |
| Sex | 0.387 (-0.496 – 1.424) | 2.691 (-0.443 – 4.977) |
| Sampling Date | 0.046 (-0.028 – 0.132) | 0.146 (-0.130 – 0.336) |

Table S3. Summary of estimates from models with various fixed effects. The final model included in the main text is in bold.

| | DIC | Baseline CORT | | Stress-Induced CORT | | Genetic Correlation (95% BCI) |
|---|---|---|---|---|---|---|
| | | $h^2$ (95% BCI) | $e^2$ (95% BCI) | $h^2$ (95% BCI) | $e^2$ (95% BCI) | |
| Intercept | 1079.848 | 0.162 (0.045 - 0.459) | 0.559 (0.282 - 0.737) | 0.345 (0.139 - 0.6) | 0.499 (0.254 - 0.688) | -0.22 (-0.786 - 0.326) |
| Intercept + Date | 1081.652 | 0.125 (0.051 - 0.471) | 0.509 (0.257 - 0.722) | 0.347 (0.129 - 0.608) | 0.476 (0.227 - 0.685) | -0.365 (-0.766 - 0.362) |
| Intercept + Date + Mass | 1071.157 | 0.207 (0.065 - 0.526) | 0.439 (0.179 - 0.642) | 0.308 (0.137 - 0.614) | 0.483 (0.225 - 0.686) | -0.351 (-0.72 - 0.384) |
| Intercept + Date + Latency Time | 1045.971 | 0.16 (0.046 - 0.393) | 0.685 (0.407 - 0.816) | 0.368 (0.138 - 0.619) | 0.474 (0.226 - 0.692) | -0.213 (-0.722 - 0.295) |
| Intercept + Date + Mass + Latency Time | 1040.488 | 0.17 (0.076 - 0.481) | 0.567 (0.283 - 0.747) | 0.357 (0.132 - 0.611) | 0.499 (0.245 - 0.7) | -0.226 (-0.691 - 0.337) |
| Intercept + Date + Mass + Latency Time + Sex | 1037.609 | 0.206 (0.071 - 0.472) | 0.552 (0.287 - 0.752) | 0.311 (0.126 - 0.602) | 0.539 (0.244 - 0.699) | -0.204 (-0.686 - 0.318) |
| Intercept + Date + Mass + Brood Size | 1070.67 | 0.22 (0.062 - 0.536) | 0.438 (0.183 - 0.654) | 0.289 (0.131 - 0.596) | 0.514 (0.25 - 0.695) | -0.359 (-0.723 - 0.39) |
| Intercept + Date + Latency Time + Brood Size | 1069.56 | 0.207 (0.066 - 0.532) | 0.374 (0.181 - 0.642) | 0.346 (0.12 - 0.592) | 0.495 (0.258 - 0.72) | -0.202 (-0.693 - 0.428) |
| Intercept + Date + Mass + Latency Time + Brood Size | 1041.032 | 0.161 (0.068 - 0.47) | 0.562 (0.299 - 0.772) | 0.325 (0.118 - 0.584) | 0.482 (0.264 - 0.716) | -0.298 (-0.69 - 0.35) |
| Intercept + Date + Mass + Latency Time + Brood Size + Sex | 1037.216 | 0.183 (0.061 - 0.462) | 0.617 (0.311 - 0.769) | 0.366 (0.125 - 0.588) | 0.492 (0.268 - 0.712) | -0.252 (-0.709 - 0.315) |
| **Intercept + Mass + Latency Time** | **1039.088** | **0.152 (0.062 - 0.458)** | **0.555 (0.309 - 0.771)** | **0.343 (0.128 - 0.598)** | **0.491 (0.251 - 0.693)** | **-0.297 (-0.688 - 0.333)** |

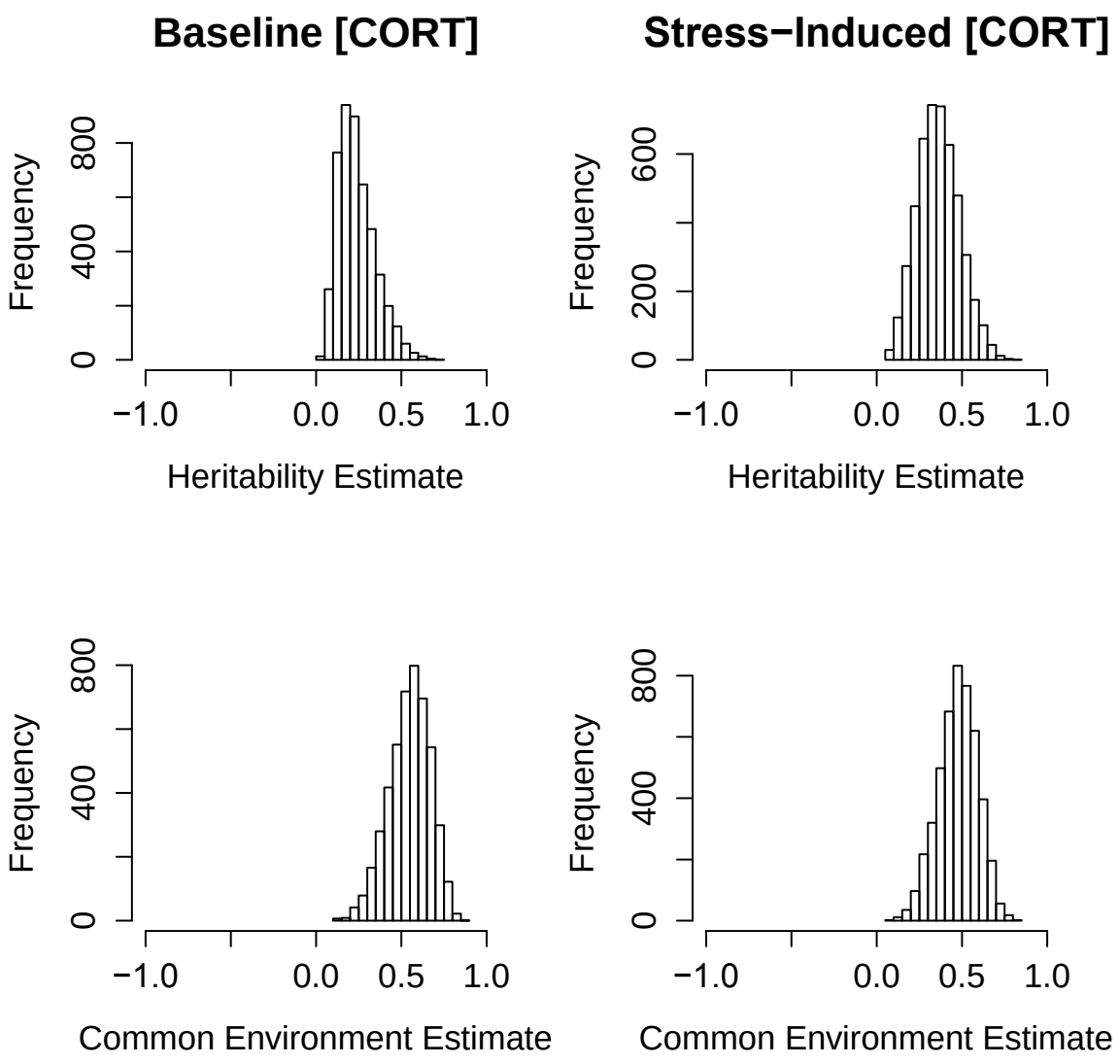


Figure S1. Posterior distributions for $h^2$ and $e^2$ estimates for baseline and stress-induced CORT measures. None of the variance ratio estimate distributions overlap zero as variance components are bound to be positive given appropriately specified priors (here $V_P$ is split evenly). *[CORT] stands for corticosterone concentration.

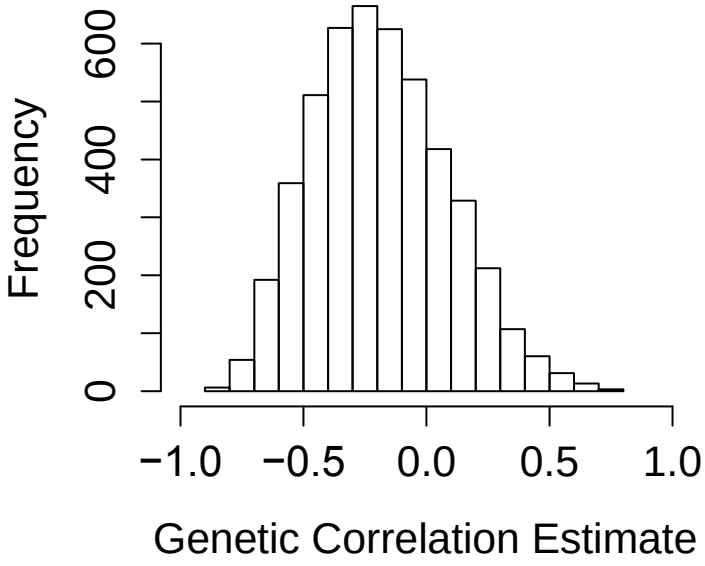


Figure S2. Posterior distributions of the estimate for genetic correlation between baseline and stress-induced CORT concentrations. The distribution overlaps zero.

References

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