**Supplementary materials for:**

**Pre- and post-copulatory traits working in concert: Sexual dichromatism in passerines is associated with sperm morphology.**

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**Methods**

*Sperm morphometric data*

Sperm from male passerines in breeding condition was collected as described in Immler et al. (2011) and processed as described in Immler and Birkhead (2005). Sperm were collected via three methods: from faecal samples (Immler and Birkhead 2005), via cloacal massage (Wolfson 1952) and directly from the seminal glomera of dissected birds (Immler et al. 2011). Sperm head, midpiece and tail lengths (±0.1µm) were measured and total length of a spermatozoon was calculated by summing these three measurements (Laskemoen et al. 2013). Where data were available for a single species from both published sources, we averaged the measurement values and used those in analyses. Sperm collected with these methods is not generally thought to significantly differ in morphometry (Immler and Birkhead 2005, but see Girndt et al. 2017). Testes mass data consisted of combined testes mass estimates taken from the literature, museum specimens and males dissected under licence as presented in Immler et al. (2011) and Rowe et al. (2015).

*Plumage dichromatism measures*

Dale et al. (2015) analysed the colours in RGB space of several plumage patches from illustrations in the Handbook of the Birds of the World (del Hoyo et al. 2003-2011), and scored each sex in each species according to the percentage of birds with similar plumage characteristics which were male. Hence, high plumage scores for males (or females) reflected “elaborate” plumage, and the difference between the scores for males and females of the same species indicated the difference in elaboration, which was interpreted as dichromatism. Dunn et al. (2015) took spectrophotometric reflectance measurements directly from feathers from several parts of the bodies of museum specimens, and conducted Principal Components Analysis on the results to extract two derived variables, which they interpreted as “brightness” and “hue”. The difference in these two variables between males and females provided two measures of dichromatism for each body part. The authors took the sum each of these measures across body parts for each species as a composite dichromatism score (henceforth referred to as “signed dichromatism”). Hence, if the male of a species was brighter or had more positive hue (more longer wavelengths) in total than the female, across the sampled body parts, the species was considered to be “dichromatic”, whilst species in which the difference was the other way around were considered not to be dichromatic, even if the difference was large. Species in which sexes were identical received an intermediate dichromatism score under this system. An alternative measure of dichromatism was the absolute difference between brightness or hue values for the two sexes. This measure had the advantage that species with identical sexes have a score of zero, and species in which males are apparently sexually selected to be less bright, or to reflect shorter wavelengths of light, were recorded as being dichromatic (e.g. male backbirds, *Turdus merula*, are conspicuously jet black, whilst females are a more cryptic brown).

*Composite predictor variable details from Dale et al. (2015)*

Body size was a combined measure derived from principal components describing body mass and wing length. Tropical life history was examined due to the high number of both brightly-coloured and sexually monochromatic species (Dale et al. 2015) and the prior suggestion of less intense sexual selection in the tropics (Stutchbury and Morton 1995, 2001). The tropical life history score was a composite variable, incorporating breeding location (inside or outside the tropics), and measures of environmental stability and clutch size.

*Details of PGLS interpretation*

We constructed our models, controlling for phylogenetic relatedness, using phylogenetic generalized least squares, as implemented in the R package *caper* (Orme et al. 2018). We downloaded 100 phylogenetic trees from www.birdtree.org ([Jetz et al. 2012](#_ENREF_42)), selected using the Hackett et al. ([2008](#_ENREF_36)) ‘backbone’, and produced averaged parameter estimates, standard errors and R2 values from the analysis done using all 100 trees. As part of the PGLS calculation *caper* uses the phylogenetic signal parameter λ ([Pagel 1997](#_ENREF_62), [1999](#_ENREF_63)) as the maximum likelihood estimated multiplier of the off-diagonal elements of the variance-covariance matrix generated from the phylogeny. In cases where λ = 0 (no phylogenetic signal in residuals), results of PGLS will approximate those obtained from phylogenetically uncontrolled ordinary least squares regression, whereas λ = 1 indicates strong phylogenetic signal, and the results of PGLS will be identical to those obtained using phylogenetic independent contrasts ([Felsenstein 1985](#_ENREF_21)).

**Results**

**Table S1.** Averaged PGLS model estimates across 100 phylogenies for relationships between plumage dichromatism and sperm or testes traits in 153 bird species, with body size and tropical life history (TLH) as covariates. Plumage dichromatism is described using two principle components (PC1, interpreted as brightness, and PC2, interpreted as hue) calculated from spectrophotometric measurements of museum specimens, taken from Dunn et al. (2015). Dichromatism was calculated in two ways for each species: the signed and the absolute (un-signed) sum of the differences in PC scores across several plumage patches. SE = Standard error. Significant results indicated in bold.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Signed dichromatism** | **Un-signed dichromatism** |
| **Response variable** | **Coefficient** | **Estimate (SE)** | **t** | **P** | **Estimate (SE)** | **t** | **P** |
|  |  | *R2 = 9.91%; λ = 1.000* | *R2 = 10.11%; λ = 1.000* |
| **Midpiece**  | PC1 | -0.003 (0.005) | -0.502 | 0.616 | 0.005 (0.027) | 0.173 | 0.863 |
| **length** | PC2 | 0.010 (0.007) | 1.290 | 0.199 | 0.042 (0.030) | 1.414 | 0.160 |
|  | Body size | -0.202 (0.062) | -3.244 | **0.002** | -0.205 (0.062) | -3.287 | **0.001** |
|  | TLH | -0.090 (0.065) | -1.399 | 0.164 | -0.085 (0.062) | -1.363 | 0.175 |
|  | Intercept | -1.319 (0.400) | -3.300 | **0.001** | -1.294 (0.399) | -3.242 | **0.002** |
|  |  | *R2 = 6.64%; λ = 0.927* | *R2 = 6.43%; λ = 0.929* |
| **Head length** | PC1 | -0.004 (0.010) | -0.367 | 0.714 | -0.020 (0.056) | -0.360 | 0.719 |
|  | PC2 | 0.000 (0.015) | 0.000 | 1.000 | 0.000 (0.058) | 0.000 | 1.000 |
|  | Body size | -0.288 (0.106) | -2.723 | **0.007** | -0.283 (0.106) | -2.662 | **0.009** |
|  | TLH | -0.119 (0.120) | -0.986 | 0.326 | -0.138 (0.118) | -1.177 | 0.241 |
|  | Intercept | 0.170 (0.600) | 0.284 | 0.777 | 0.151 (0.605) | 0.250 | 0.803 |
|  |  | *R2 = 3.06%; λ = 0.964* | *R2 = 3.42%; λ = 0.961* |
| **Tail length** | PC1 | 0.003 (0.006) | 0.457 | 0.648 | -0.005 (0.037) | -0.127 | 0.899 |
|  | PC2 | -0.005 (0.009) | -0.572 | 0.568 | 0.039 (0.038) | 1.022 | 0.308 |
|  | Body size | 0.146 (0.074) | 1.964 | 0.051 | 0.146 (0.074) | 1.970 | 0.051 |
|  | TLH | -0.031 (0.082) | -0.383 | 0.703 | -0.042 (0.079) | -0.528 | 0.528 |
|  | Intercept | 0.998 (0.445) | 2.241 | **0.027** | 0.988 (0.441) | 2.240 | **0.027** |
|  |  | *R2 = 10.41%; λ = 1.000* | *R2 = 11.24%; λ = 1.000* |
| **Total sperm**  | PC1 | -0.002 (0.006) | -0.320 | 0.749 | 0.001 (0.031) | 0.029 | 0.977 |
| **length** | PC2 | 0.010 (0.009) | 1.123 | 0.263 | 0.056 (0.034) | 1.629 | 0.105 |
|  | Body size | -0.249 (0.072) | -3.438 | **0.001** | -0.252 (0.072) | -3.493 | **0.001** |
|  | TLH | -0.102 (0.075) | -1.358 | 0.177 | -0.099 (0.072) | -1.366 | 0.174 |
|  | Intercept | -1.150 (0.464) | -2.478 | **0.014** | -1.124 (0.462) | -2.434 | **0.016** |
|  |  | *R2 = 45.21%; λ = 0.660* | *R2 = 44.46%; λ = 0.658* |
| **Testes mass** | PC1 | 0.012 (0.008) | 1.496 | 0.137 | 0.056 (0.052) | 1.079 | 0.282 |
|  | PC2 | 0.013 (0.013) | 1.046 | 0.297 | 0.046 (0.052) | 0.884 | 0.378 |
|  | Body size | 0.789 (0.075) | 10.523 | **<0.001** | 0.795 (0.076) | 10.517 | **<0.001** |
|  | TLH | -0.065 (0.100) | -0.645 | 0.520 | -0.111 (0.099) | -1.124 | 0.263 |
|  | Intercept | -0.038 (0.338) | -0.112 | 0.911 | -0.052 (0.337) | -0.153 | 0.879 |

**Table S2.** Averaged PGLS model estimates across 100 phylogenies for relationships between Dichromatism (sex difference in colour elaboration taken from Dale et al. 2015) and sperm traits in birds, with body size and tropical life history as covariates, using only the 153 bird species that were also included in a different analysis of plumage colour (Dunn et al., 2015). SE = Standard error. Significant results indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Response variable** | **Coefficient** | **Estimate (SE)** | **t** | **P** |
| **Midpiece length** | Dichromatism | 0.046 (0.034) | 1.329 | 0.186 |
| *R2 = 9.91%,*  | Body size | -0.208 (0.062) | -3.345 | **0.001** |
| *λ = 1.000* | Tropical life history | -0.085 (0.062) | -1.373 | 0.172 |
|  | Intercept | -1.285 (0.398) | -3.226 | **0.002** |
| **Head length** | Dichromatism | 0.048 (0.067) | 0.713 | 0.477 |
| *R2 = 6.74%,*  | Body size | -0.288 (0.105) | -2.741 | **0.007** |
| *λ = 0.923*  | Tropical life history | -0.145 (0.117) | -1.240 | 0.217 |
|  | Intercept | 0.163 (0.595) | 0.274 | 0.785 |
| **Tail length** | Dichromatism | 0.026 (0.044) | 0.578 | 0.564 |
| *R2 = 2.99%,*  | Body size | 0.147 (0.074) | 1.990 | **0.048** |
| *λ = 0.958* | Tropical life history | -0.040 (0.079) | -0.510 | 0.611 |
|  | Intercept | 0.993 (0.437) | 2.272 | **0.025** |
| **Total sperm length** | Dichromatism | 0.055 (0.040) | 1.389 | 0.167 |
| *R2 = 10.77%,*  | Body size | -0.256 (0.072) | -3.541 | **0.001** |
| *λ = 1.000* | Tropical life history | -0.099 (0.072) | -1.372 | 0.172 |
|  | Intercept | -1.113 (0.463) | -2.402 | **0.018** |
| **Testes mass** | Dichromatism | 0.015 (0.058) | 0.291 | 0.772 |
| *R2 = 43.16%,*  | Body size | 0.790 (0.076) | 10.333 | **<0.001** |
| *λ = 0.673*  | Tropical life history | -0.101 (0.099) | -1.019 | 0.310 |
|  | Intercept | -0.052 (0.345) | -0.150 | 0.881 |

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