**Supplementary Material from “Social plasticity and individuality shape variation in contest behaviour”**

***1.1 Morphological measurements***

To measure body and foreleg sizes of *Drosophila prolongata* males, individuals were first euthanised by freezing at –20°C, thawed, and their left foreleg was removed under a stereomicroscope. The body was mounted on its right side and the foreleg on separate microscope slides using instant adhesive (Pattex® Henkel AG & Co., Düsseldorf, Germany). The foreleg and body were photographed using a camera setup mounted on a Zeiss stereomicroscope (Carl Zeiss Microscopy GmbH, Jena, Germany). From these photographs, we measured foreleg femur length and width, foreleg tibia length, first tarsal segment length, and thorax length (distance from the tip of the scutellum and the base of the head) using ImageJ version 1.53h (https://imagej.net). Thorax length is a widely used proxy of body size in drosophilids (Rohner et al. 2018). We note that adult body size does not vary with age as it is fixed at the end of the larval development, as in any other holometabolous insects (Hanna et al. 2023).

***1.2 Details of statistical analyses***

In addition to assessing trait variability (see Methods in the main text), we estimated pairwise Pearson’s correlations among morphological traits and a repeated measures correlation between territoriality and aggressiveness (Bakdash and Marusich, 2017). All foreleg traits were highly positively correlated but were only weakly correlated with thorax length (Figure S1). Thus, to avoid collinearity among fixed effects in linear mixed models, we conducted subsequent analyses using tibia length as a measure of foreleg size and thorax length as a measure of body size. Selecting tibia length as a surrogate for foreleg size was based on the results of a principal component analysis (PCA) revealing that the primary axis of morphological variation among individuals corresponded mainly to variation in tibia length (Table S1). Behavioural traits were not significantly correlated (see Results: Morphological and behavioural variation) and were therefore analysed as independent variables. This is also justify given that the two traits are functionally distinct. Territorial success can arise from direct aggression or be established without contact, through threat displays or other visual cues of competitive ability (e.g. body size). In contrast, aggressiveness reflects a behavioural tendency not always linked to competitive ability: small males often fight intensely against size-matched rivals, whereas large males may show little aggression when facing smaller opponents. Yet, large males typically achieve higher resource occupancy (De Nardo et al. 2025).

To examine the sources of variation in contest behaviour, we calculated the marginal 𝑅2 and the conditional *R2* for each model, which provide a measure of the variance explained by the fixed effects and random effects (Nakagawa and Schielzeth 2013). The marginal 𝑅2 quantified the proportion of variation in contest behaviours arising from morphological variation in body size and foreleg size of focal males and their opponents. Meanwhile, the conditional 𝑅2 quantified unexplained variation in contest behaviours arising from individual differences among focal males and opponents in other (unmeasured) traits, as well as due to the specific day and time the behaviour was measured. The marginal *R2* and conditional *R2* were computed using the *r2\_bayes* function of the R package *performance* (Lüdecke et al. 2021).

We then evaluated the relative proportion of variance in contest behaviour arising from consistent individual differences in these traits. To this end, we calculated the repeatability (*R*) of territoriality and aggressiveness using variance estimates provided by each model as

where *VFocal* is the variance arising from differences among focal males, *VOpponent* the variance arising from opponent identity, *Vdate* and *VTime* the variances arising from the specific calendar date and time at which the behavioural assay was conducted, respectively, and *Vresidual* the residual variance. To calculate repeatability on the latent (logit) scale, we used the latent residual variance (Nakagawa and Schielzeth 2010), which is fixed and can be calculated as . Lastly, we assessed the relative proportion of variance in focal contest behaviour arising from consistent individual differences among opponents using the above formula and replacing *VFocal* by *VOpponent* in the numerator.



**Figure S1.** Matrix of correlations among morphological traits. Data were obtained from 52 males.

**Table S1:** Loadings of the first principal component (PC) reflecting combinations of foreleg traits.

|  |  |  |
| --- | --- | --- |
| Trait |  | PC1 |
| Femur length |  | 0.475 |
| Tibia length |  | 0.511 |
| Tarsus length |  | 0.304 |
| Femur width |  | 0.486 |
| Tibia width |  | 0.426 |

**Table S2:** Descriptive statistics of morphological traits calculated from unscaled values measured on 52 males for morphological traits, and on 53 males for behavioural traits. Morphological traits are in mm and behavioural traits represent a number of minutes (scans) out a total of a period of 30 minutes during which behaviours were recorded.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Trait |  | Average | St. Dev. | Minimum | Maximum | CV (%) |
| Morphologies |  |  |  |  |  |  |
| Femur length |  | 1.46 | 0.089 | 1.26 | 1.69 | 6.14 |
| Femur width |  | 0.588 | 0.034 | 0.521 | 0.693 | 5.79 |
| Tibia length |  | 1.43 | 0.077 | 1.31 | 1.62 | 5.39 |
| Tibia width |  | 0.323 | 0.036 | 0.254 | 0.424 | 11.1 |
| Tarsus length |  | 0.521 | 0.041 | 0.430 | 0.588 | 8.02 |
| Thorax length |  | 1.97 | 0.138 | 1.68 | 2.19 | 6.99 |
| Behaviours |  |  |  |  |  |  |
| Territoriality |  | 16.1 | 8.81 | 0 | 30 | 54.6 |
| Aggressiveness |  | 4.22 | 4.46 | 0 | 20 | 105.5 |



**Figure S2.** Effects of foreleg size in opponents (tibia length) and focal body size (thorax length; A), opponent foreleg and body sizes (B), and (C) focal foreleg and body size on territoriality.

**Table S3:** Variance estimates from Bayesian linear mixed-effects models analysing territoriality and aggressiveness. Means represent point mode estimates with 95% credible intervals.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | Territoriality | Aggressiveness |
| Variance component |  | Parameter | Mean (± 95% CI) | Mean (± 95% CI) |
| Focal ID |  | *VFocal* | 1.20 (0.499–2.44) | 1.27 (0.489–2.63) |
| Opponent ID |  | *VOpponent* | 0.473 (0.113–1.19) | 0.984 (0.329–2.21) |
| Date |  | *VDate* | 1.23 (0.004–5.037) | 0.830 (0.104–2.80) |
| Time |  | *VTime* | 1.15 (0.254–2.98) | 0.851 (0.018–2.84) |
| Residual |  | *VResidual* | π2/3 | π2/3 |

***1.3 Coefficients of variations in morphological traits***

We found that variability in thorax and foreleg length was comparable to that in previous studies on *Drosophila prolongata*. Using raw measurements reported by Perdigón Ferreira et al. (2023) for 506 males across 21 isofemale lines, we calculated coefficients of variation (CVs) of 8.53% for thorax length and 7.63% for foreleg tibia length. From data presented by Perdigón Ferreira and Lüpold (2022) for 240 males from an outbred population, we obtained CVs of 4.24% for thorax length and 4.22% for foreleg length (sum of the femur, tibia, and first tarsal segment). Overall, these values are relatively high compared with CVs reported from *Drosophila melanogaster* (e.g. Coyne and Beecham 1987; Imasheva et al. 1994; Morteau et al. 1995; Karan et al. 1999). For example, in isofemale lines of *D. melanogaster* Karan et al. (1999) reported CVs of about 1.4% lines for thorax length. In outbred populations of *D. melanogaster* reared in the laboratory, CV approached 2.0% for thorax length (Morteau et al. 1995; Karan et al 1999) and 4.5% for wing length (Imasheva et al. 1994). It is possible that the relative high CVs in our study reflect species specific variability and that morphological traits are more condition-dependent in *D. prolongata*.

***1.4 Correlations among morphological traits and between behavioural traits***

Not surprisingly, we found positive correlations among all leg segments (Figure S1). Length and width measurements were moderately to strongly correlated for the tibia and femur, indicating possible isometry among these segments. Tarsus length was positively correlated with femur and tibia sizes, although to a lesser extent. In contrast, correlations between thorax length and the size of the different leg segments were weak (i.e. ≤ 0.25), indicating a possible allometry between body size and foreleg length. To avoid collinearity among fixed effects in subsequent analyses, tibia length and thorax length were the sole morphological traits used to test for effects of foreleg size and body size. Lastly, there was no evidence for a correlation between territoriality and aggressiveness (r = 0.178, CI = −0.020–0.364, df = 148, P = 0.078).

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