**The evolution of sexual dimorphism and condition dependence in *Drosophila prolongata***

**Background**

The evolution of differing form and function in traits expressed in both sexes, called sexual dimorphism, provides a framework for studying the evolutionary and developmental mechanisms that underlie within-species variation.

Sex-specific trait expression often manifests as male trait exaggeration (Emlen, 2008). Strong sexual selection on males, via inter- and intrasexual competition tends to favour exaggerated traits (i.e.., traits that scale disproportionately with body size). Despite their advantage in sexual competition, these traits are energetically costly to express. Therefore, the evolution of sexually selected traits my reflect a trade-off between the cost of exaggerated trait expression and its advantage in sexual competition (Rowe and Houle, 1996). Theory predicts that by co-evolving a form of developmental plasticity called condition-dependence with the sexually dimorphic traits, individuals can optimize the benefit of exaggerated trait expression. Males with greater access to metabolic resources (i.e., in good condition), will grow to be larger, and larger males will allocate more resources to traits that accrue benefits in sexual competition while still incurring an equivalent cost of trait exaggeration as low condition individuals (Bonduriansky, 2007). Traits that are subject to stronger directional sexual selection should evolve heightened condition dependence. Therefore, strong sexual selection on exaggerated male traits should give rise to the evolution of male-biased condition dependent expression.

*Drosophila prolongata* has evolved novel sex-specific traits and behaviours, providing an interesting framework for understanding how and why sexual dimorphism evolves. Unlike *D. melanogaster*, and most of the *melanogaster* species group, *D. prolongata* exhibits a reversal in sexual (body) size dimorphism, with males being the larger sex (Leucke and Kopp, 2019; Setoguchi et al., 2014). Furthermore, D. *prolongata* males express exaggerated, patterned forelegs. These exaggerated forelegs are associated with male-male combat for access to resources, and a novel mating behaviour called leg vibration, which increases female receptivity to mating, suggesting that the evolutionary trajectory of the trait is determined by sexual selection (Setoguchi et al., 2014; Toyoshima & Matsuo, 2023).

**Hypothesis**

Males with greater access to metabolic resources will allocate more resources to the expression of exaggerated traits, while incurring an equivalent cost of sexually dimorphic trait expression, relative to males in ‘poor’ condition (i.e., those having limited access to metabolic resources). Sexually dimorphic trait expression should co-evolve with the evolution of condition dependent trait expression. Traits that are subject to stronger directional sexual selection will evolve to be more condition dependent.

**Predictions**

I.Reducing access tometabolic resources during the period of trait development will reduce foreleg size in male*D.prolongata*, reducing the extent of sexual dimorphism in the trait (i.e., the degree that the male and female phenotype differs)

II. The sexually selected forelegs of male *D.prolongata* are subject to more intense directional sexual selection and so should exhibit heightened condition-dependence relative to the wings. Reducing environmental components of condition during larval development should result in a greater reduction in the extent of sexual dimorphism in the foreleg than the wings.

**Methods**

*Nutritional manipulation*

The nutritional manipulation experiment was designed and carried out by Dr. Maria Pesevski and Dr. Ian Dworkin (Pesevski, 2021). Data was collected by Dr. Maria Pesevski.

To manipulate environmental components of condition, flies were subject to increasing periods of starvation during larval development. Diet manipulation during larval development (prior to the third larval instar) reduces absolute and relative trait size in adult *Drosophila* (Stillwell et al. 2011). Flies in cohort 1 were fully fed, and each subsequent cohort level was starved for one day (24 hours) longer than the preceding cohort. Flies were starved up for up to 72 hours (cohort 4).

After the nutrition manipulation, the right wing and right foreleg of 30 adult males and 30 adult females were dissected and imaged. Linear measurements (in millimeters) of the thorax, tibia length and width, and length of the first tarsal segment were taken. Measurements of wing area were also taken.

*Data preparation and clean-up*

The original data set containing data from 27 species was subsetted to create a data frame containing only values for *Drosophila prolongata.* The *D.prolongata* data frame consists of 81 observations: 46 females (17 high condition; 29 low condition) and 35 males (22 high condition; 13 low condition). Fully fed flies (cohort 1) were coded as high condition (HC) and 72-hour starved (cohort 4) flies were coded as low condition (LC). All raw leg trait values (tibia length and width, tarsus length) and thorax length were converted to micrometers (x 1000) and log2 transformed. The purpose of the log2 transformation was so standardize trait values, allowing for comparison between traits and to allow us to frame our interpretation in term of proportional changes between traits during our analysis. The conversion to micrometers was to avoid computing negative log values, in order to simplify our interpretation of treatment contrasts.

Sex was coded factor with ordered levels “F”, “M” (female and male, respectively). Condition was coded as a factor with ordered levels “HC”, “LC”. We coded “HC” as the base level such that we can interpret changes in our response at the low condition (starved) state relative to the high condition (fully-fed) state when modeling condition as predictor variable.

***Statistical analysis: Multivariate mixed effects model***

The test the hypothesis that depriving flies of nutrition during larval development will reduce the extent of sexual dimorphism in the forelegs, we modeled the effect of condition and sex and their interaction on our 3 foreleg traits and thorax size (as a measure of body size) and controlled for individual differences between specimens using a multivariate mixed effects model.

We modelled the effect of sex and condition (and their interaction) on the size of our three foreleg traits (tibia and width, tarsus length) and body size (i.e., thorax length) using a multivariate response model to allow for correlation among our four traits. We included body size (i.e., thorax size) as response variable to allow for comparison between the effect of variables of interest on foreleg size and body size. The exaggerated forelegs should scale disproportionately with body size, we therefore expect that limiting access to nutrition will yield a reduction in leg size that is disproportionate to a change in body size. We were therefore interested in differentiating between the effect of our nutritional manipulation on sexual dimorphism in body size and foreleg size.

The multivariate model was fit using the *lmer* function from the *lme4* package.

*Converting our data frame to the long format*

To fit our multivariate model using *lmer*, we generated a long format of our original data frame, where each trait represents a repeated measure within a single column. The column, *value*, stored length measurements for each response variable (i.e., trait: tibia length, tibia width, tarsus length, thorax length). The column, *trait*, stores the name of each response variable. We created a column, *units*, which assigned a value to each individual (n = 81) in the data set. This variable was used in our random effects formula to control for variation between individuals in the data set.

*Choice of fixed and random effects*

Fixed effects formula: trait:(sex \* condition) – 1

We were interested in modelling the effect of sex, condition, and their interaction, on our foreleg traits and body size to ask how 1) traits differ in the extent of sexual dimorphism, 2) in how much they differ in their response to a reduction in condition, and how the reduction in the extent of sexual dimorphism in response to starvation differs across traits, respectively.

Trait is included as a predictor variable to allow the model to evaluate the effect of the variables of interest (sex and condition) on each trait, where the variable, *trait*, represents a repeated measures of each leg and thorax measurement from each fly.

The intercept was supressed to prevent the model from having the traits interact with one another.

Random-effects formula: (trait-1|units)

By including *unit* as a random effect, we are estimating the mean and distribution of individual effects on each of our traits. Generates the residual variance-covariance matrix among individuals for each trait. The residual variance-covariance matrix among individuals for each trait, suggests that there is a high correlation among individuals for each trait comparison, suggesting that most of the variation among these traits is due to size and unlikely that individual effects had a large, or biologically relevant effect on our trait measurements.

*Diagnostics*

We used the *simulateResiduals* function from the *DHARMa* package, the *check\_model* function from the *performance* package*,* and the *qqmath* function from the *lattice* package to check the fit of our model.

The diagnostic plots generated using check\_model mostly suggested that the model had been correctly specified. We did not observe any notable deviations from assumptions of linearity and homoscedasticity of the residuals or high leverage residuals. The plot for normality of residuals generates a sloped line, indicating that our residuals deviate from assumptions of normality.

However, because the pattern of the points along the QQ plot seemed unusual, we generated QQ plots to better identify the pattern of non-normality of residuals.

QQ plot generated using the *simulateResiduals* function using suggests that the residuals are skewed to the left. The QQ plot generated using the *qqmath* function also confirms that the residuals are underestimated in the first quantile.

To further confirm that our model fit was correctly specified, we ran the allFit() function to refit the models using different optimizers. All optimizers provided nearly identical parameter values. Taken together, we concluded that the model fit was reasonable enough to proceed.

*Multivariate single effect linear model*

We were unable to generate treatment contrasts for sex using the model run in *lmer*.To properly evaluate the effect of sex and the interaction between sex and condition, on our trait sizes, we dropped the random effect formula and ran the same model (i.e., identical fixed effects) as a multivariate linear model using the *lm* function from the *stats* package. We used this linear model to generate coefficient plots, and treatment contrasts using *emmeans*.

*Interpretation, Coefficient plots, Estimated marginal means and contrasts*

We used the *emmeans* and *contrast* functions from the *emmeans* package to generate interaction contrasts between the sex and condition levels.

While we were unable to generate treatment contrasts from our multivariate mixed effects model, we were able to generate contrasts using *emmeans*. The contrasts produced by *emmeans*, appeared to be reasonable, given the values imputed into the model. To confirm that the treatment contrasts made biological sense, we cross-referenced the estimates produced by *emmeans* using this model with those generated using an identical multivariate model using *lm* (i.e., after removing our random effects). Both models produced similar (but not identical) estimates for corresponding contrasts) (See figures 2 and 3). However, the confidence intervals (CIs) deviated between both models, producing CIs that did not cross zero and CIs that cross zero in the mixed effect and fixed effect models, respectively.

**Results**

*Starvation during larval development does not decrease the extent of sexual dimorphism in the sexually selected forelegs.*

Our starvation treatment did reduce overall body size and foreleg size. However, the foreleg traits were not reduced in a manner that was disproportionate to the reduction in body size, as expected. The effect of condition on all four traits was negative and the direction and magnitude of the effect was similar across all four traits (Figure 1). All four traits exhibited male-biased sexual dimorphism, as indicated by the positive effect of sex. Sex was the dominating effect on all traits, except thorax size, where magnitude of the condition effect was larger. Condition did not have a biologically relevant effect on the direction of sexual dimorphism. The effect of the interaction between condition and sex on all four traits was negative (but nearly zero) and non-significant.

Using the mixed effects linear model, we generated two-way interaction treatment contrasts between sex and condition to determine the magnitude of the reduction in sexual size dimorphism (SSD) (i.e., difference in size between males and females) across our across our leg traits, and between our leg traits and body size. We found that the magnitude of the reduction in SSD due to our starvation treatment was not biologically relevant. We also did not find a relevant difference in magnitude or direction of change in SSD in our foreleg traits and thorax size, suggesting that the forelegs and body respond similarly to nutritional manipulation. The change in sexual dimorphism in tarsus length was not statistically significant (Figure 2). Change in SSD in the remaining traits were also very close to zero. The change in sexual size dimorphism across condition treatments was nearly identical in tibia length and tarsus length.

Our two-way interaction contrasts using our linear model confirm the pattern observed in the previous analysis, however, all confidence include zero, suggesting that the differences are not biologically relevant. Across all traits, the change in the extent of sexual dimorphism due to our starvation treatment is negative and non-significant (Figure 3). Overlapping confidence intervals suggest that the effect of condition on sexual dimorphism is similar in all four of our traits, with the smallest reduction being in tarsus length.

To determine if the expression of condition dependence is sexually dimorphic, we looked at the how the difference in condition (i.e., high condition vs low condition) differed between males and females. The reduction in condition yielded a greater change in size in males than females for tibia length, tibia width, thorax length (Figure 4). The change in tarsus length for males and females is nearly identical. Condition treatment confidence intervals for each sex overlap for trait overlap, suggesting that the difference is not biologically relevant.

A graph of different sizes of a person's body

Description automatically generated with medium confidence

Figure 1. The effect of sex, condition, and their interaction on foreleg and boy size.

95% confidence intervals are shown. All responses variables have been log2 transformed.

A graph of a number of different types of data

Description automatically generated with medium confidence

Figure 2: Log2 change in SSD at HC vs LC using the multivariate mixed model in lmer. Bars show 95% confidence intervals.

A graph of a graph with a number of lines

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Figure 3. Two-way interaction contrasts showing Log2 change in SSD at High Condition (HC) and Low condition (LC) in 3 foreleg traits and body size. Bars show 95% confidence intervals.

A graph of a number of objects

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Figure 4. Log2 change in trait size for at high condition (HC) vs low condition (LC) in males (M) and females (F) in foreleg traits and body size.

**Discussion**

**Biological interpretation**

Our results therefore suggest that the expression of sexual size dimorphism in the forelegs of *Drosophila prolongata* likely do not respond to a reduction in condition during larval development in a manner that is biologically relevant.

*Future statistical steps*

*References*

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