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

**Background**

The evolution of divergent 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 reflects a trade-off between the cost of exaggerated trait expression and its advantage in sexual competition (Rowe and Houle, 1996). Theory predicts that by expressing a form of developmental plasticity called condition-dependence in the sexually selected trait, individuals can optimize the benefit of exaggerated trait growth, allowing for further evolutionary response (i.e., trait exaggeration). 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 dependence. The relationship between sexually dimorphic trait expression and condition dependence has been demonstrated in several species (Zinna et al., 2014; Oudin et al. 2015), including *Drosophila* (Rohner and Blackenhorn, 2018).

*Drosophila prolongata* has evolved a suite of 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. 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 (Setoguchi et al., 2014; Amino and Matsuo, 2023).

**Hypothesis**

By evolving condition-dependent expression for sexually selected traits, individuals will optimize the trade-off between the advantage accrued in sexual competition vs the viability cost of expressing an energetically costly trait. Males with greater access to metabolic resources will be able to allocate more resources to the expression of exaggerated traits, while accruing an equivalent cost of sexually dimorphic trait expression, relative to males in ‘poor’ condition (i.e., having limited access to metabolic resources). Sexual selection will favour further trait exaggeration (i.e., disproportionate growth of the trait relative to the body). Traits that are subject to stronger directional sexual selection will evolve to be more condition dependent.

**Predictions**

I.Reducing access tometabolic resources during the critical period of trait development will reduce the extent of exaggerated foreleg growth in male*D.prolongata*.

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.

**Methods**

*Nutritional manipulation*

The nutritional manipulation experiment was designed and carried out by Dr. Maria Pesevski and D. Ian Dworkin. Data was collected by Dr. Maria Pesevski.

To manipulate environmental components of condition, flies were subject to increasing periods of starvation during larval development, the critical period for organ development in *Drosophila*. 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 dataframe containing only values for *Drosophila prolongata. D.prolongata* data frame contains 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). 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 values 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.

Sex is ordered “F”, “M”. Condition is ordered “HC”, “LC”. “HC” will be the base level to facilitate contrasts that evaluate high condition (fully-fed) state vs low condition (starved) when modeling condition as predictor in future analyses.

***Multivariate mixed effects model***

To test the prediction that reducing environmental condition during larval development reduces the extent of sexually dimorphic foreleg exaggeration in male *D. prolongata*, 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.

*Choosing a multivariate model*

We modelled the effect of sex and condition (and their interaction) on size of our three foreleg traits and body size (i.e., thorax length) using a multivariate model to allow for correlation among our traits (i.e., leg traits and body size).

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

*Converting to our data frame to the long format*

To fit the multivariate model using lmer, our data frame was first converted to a ‘long’ format, to fit a pseudo univariate model 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; all measurements have been log2 transformed). The column, *trait*, stores the name of each response variable. We also created a column, *units*, which assigned a value to each individual (n = 81). in the data set. This variable was for use in our random effects formula to account for variation between individuals in the data set.

*Choice of fixed and random effects*

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

Trait is included as a predictor variable to allow the model to evaluate the effect of the predictors I am interested in (sex and condition) on each trait, where trait represents repeated measures of each leg and thorax measurement from a single fly.

The intercept is removed to prevent the model from having each trait interact with each other.

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

Generates the variance-covariance matrix among individuals for each trait. The VCV 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 shape. Tibia width is the least correlated among the other length measurements among individuals.

I would have liked to include a nested model, to look at the variation between individuals in each condition cohort (i.e., condition as a grouping variable).

*Diagnostics*

We used the *simulateResiduals* function from the DHARMa package as well as the *check\_model* function from the *performance* packageto check the fit of our model.

*Warnings and troubleshooting*

1: In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :

unable to evaluate scaled gradient

2: In checkConv(attr(opt, "derivs"), opt$par, ctrl = control$checkConv, :

Model failed to converge: degenerate Hessian with 1 negative eigenvalues

Used allFit() function from the lme4 package to try different optimizers. Al results are similar, except for the Nelder\_Mead optimizer, which produces a high negative log-likelihood relative to the best fit. All optimizers also appear to provide identical parameter values

<https://stackoverflow.com/questions/70537291/lmer-model-failed-to-converge-with-1-negative-eigenvalue> - refer for troubleshooting code

*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.