**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 co-evolving sexual dimorphic trait expression with a form of developmental plasticity called condition-dependence, individuals can optimize the benefit of exaggerated trait growth, promoting 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 majority of *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, suggesting that the evolutionary trajectory of the trait is determined by sexual selection (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 incurring an equivalent cost of sexually dimorphic trait expression, relative to males in ‘poor’ condition (i.e., those 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 foreleg size in male*D.prolongata*, reducing the extent that the male and female phenotype differs (i.e., the extent of sexual dimorphism.

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. Purpose of micrometer conversion was to avoid computing negative log values to make it easier to interpret treatment contrasts.

Sex was made a factor with ordered levels “F”, “M” (female and male, respectively). Condition was made a factor with ordered levels “HC”, “LC”. “HC” will be the base level to facilitate contrasts that evaluate 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***

To test the prediction that depriving flies of nutrition during larval development will reduce foreleg size in male*D.prolongata*, thereby reducing the extent of sexual dimorphism, 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.

*Choice of a multivariate model*

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

Body size was included as a response variable to allow for correlation between our leg traits and body size. The exaggerated trait 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 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 original length measurements taken in micrometres were converted to millimeters and log2 transformed, to standardize measurement across traits and allow for comparison of proportional changes. 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 for use 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

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 each fly.

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

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

By including unit as a random effect, we are estimating the mean and distribution of individual effects. We are allowing the effect of each individual to vary.

Generates the residual 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 size. Tibia width is the least correlated among the other measurements, which are all length 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.

Diagnostic plots generated using check\_model mostly suggest that the model has been correctly specified. I do not observe any notable deviations from assumptions of linearity and homoscedasticity of residuals or high leverage residuals. However, the plot for normality of residuals generates a sloped line, suggesting deviation from normality in our residuals.

QQ plot generated using simulated residuals *simulateResiduals* function using suggests that the residuals are mostly uniformly distributed but deviate significantly at the first quantile. The QQ plot generated using the *qqmath* function also confirms that the residuals are mostly normally distributed but are underestimated in the first quantile.

Taken together, I decided that the model fit was sufficient and proceeded.

**Note for me:**

Dharma resimulates data from the model, fit new models, calculates the residuals, then draws a QQ plot from based on simulated residuals.

*Troubleshooting*

Running the model produced the following warnings.

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 the allFit() function to refit fitted models using different optimizers. All optimizers produce, similar, negative AICs , suggesting that my model fit is sufficient. 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

*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.

Used *dwplot* function from the *dotwhisker* package to generate coefficient plots

*Multivariate single effect linear model*

We removed the random effect and ran the same model as a multivariate linear model using the *lm* function from the *stats* package. We used the linear model to generate coefficient plots, and treatment contrasts using *emmeans*.

**Results**

Multivariate mixed model using lmer()

*Interaction contrasts using the multivariate mixed model*

*Question: The effect of condition on sexual dimorphism – Does depriving flies of nutrition during larval development decreases the extent of sexual dimorphism in foreleg traits. How is the effect of starvation different in the leg traits than in body size. I expect to see a greater reduction in sexual dimorphism in the leg traits than in body size.*

Condition has a very little effect on the extent of sexual dimorphism in tarsus length. Condition interval crosses zero, so it is unlikely that the effect is biologically relevant.

Condition has the greatest effect on sexual dimorphism in tibia width.

Condition has a very similar effect on sexual dimorphism in tibia length and tarsus.

Note for me: The contrasts represent the difference in mean trait size in (females – male) at (high condition – low condition)

A graph of a comparison

Description automatically generated

*Dot-whisker plot for lmer model with random effects*

*Question: Is condition-dependence male-biased: Does depriving flies of nutrition during larval development yield a greater reduction in foreleg size in males than in females?*

*Effect of condition: change in log2 mean trait size in (high condition – low condition)*

The effect of condition is greatest on tarsus length and thorax length. Condition has a similar effect on the decrease in tarsus and thorax length.

Condition has a much smaller effect on trait size than sex.

*Effect of sex:*

*Sex: F - Change in log2 mean trait size in male – female? Does not make sense, female is the base level*

*Sex: M – mean across males and females*

*Effect of the interaction between sex and condition:*

A screenshot of a graph

Description automatically generated

Multivariate fixed effect only using lm()

*Coefficient plot*

Effect of sex is dominating effect in all traits except thorax length. Sex has the greatest effect on tibia width. Greatest amount of variation in effect size in thorax length. Effect of sex is smallest in thorax length.

The effect of the interaction between sex and condition is very small in all traits. All confidence intervals cross zero, suggesting that the effect is likely not biologically relevant.

Effect of condition is small across all traits.

A graph of different sizes of a person's body

Description automatically generated with medium confidence

*SSD contrasts: effect of condition on sexual size dimorphism*

Confidence intervals all overlap with zero, effect of condition on sexual dimorphism on all traits is not biologically relevant.

Condition dependence has smallest effect on sexual size dimorphism in tarsus length.

Condition dependence has similar effect on sexual size dimorphism in tibia length and thorax length.

A graph of a graph with a number of lines

Description automatically generated with medium confidence

*SSD contrasts: is condition dependence sexually dimorphic – is the effect of condition greater in males than females*

Condition treatment confidence intervals for each sex overlap for trait overlap – difference is not biologically relevant.

Effect of condition is greater in males than females in tibia length, tibia width, thorax length.

Effect of condition on thorax length is nearly identical in males vs females

Note to me: contrasts are the difference in mean trait size in HC – LC

*Question: The effect of sexual dimorphism on condition dependence – Does depriving flies of nutrition during larval development more significantly impact sexually dimorphic traits? How is the effect of starvation different in leg traits compared to wing area? I expect to see a greater dependence on condition in leg traits than wing area.*