**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 Therefore, the evolution of these 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). Condition-dependence couples trait expression with the genetic and environmental condition of the individual. Individuals have access to a non-replenishable pool of metabolic resources that they can allocate to all traits that enhance fitness. Males with greater access to metabolic resources, will grow to be larger, and larger males will allocate more resources to traits that accrue benefits in sexual competition while still having resources remaining for the expression of traits that increase viability fitness (Bonduriansky, 2007). Furthermore, 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).

Last having an ancestor with *Drosophila melanogaster,* 20 million years ago, *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 mates, and a novel mating behaviour called leg vibration, which increases 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 a lower 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 should exhibit heightened condition-dependence relative to the non-sexually selected 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 taken.

*Data preparation and clean-up*

The original data set containing data from 27 species was subset 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 already coded as high condition (HC). 72 hour starved (cohort 4) flies were coded as high condition. All raw values (taken in millimeters) were converted to micrometers and log2 transformed. This was to standardize values, allowing for comparison across traits.