

# 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 may 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 dependence.

*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 (Luecke and Kopp, 2019). 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

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). 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 to metabolic 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.

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 D. 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 subsetting to create a dataframe 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), wing area values, and thorax length values were converted to micrometers (x 1000, x 1 000 000 for wing area) and log2 transformed. The purpose of the log2 transformation was so standardize trait values, allowing for comparison between traits and to allow us to compare proportional changes in trait size during our analysis. Conversion to micrometers was to avoid computing negative log values, in order to simplify our interpretation of treatment contrasts.

Sex was converted to a factor with ordered levels “F”, “M” (female and male, respectively). Condition was converted to a factor with ordered levels “HC”, “LC”. We coded “HC” as the base level such that our treatment contrasts evaluate our response at 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 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.

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.

The exaggerated trait should scale disproportionately with body size, we 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, by including body size as a response variable.

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*, 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:  $\text{trait}:(\text{sex} * \text{condition}) - 1$

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 their extent of sexual dimorphism, (2) in how much they differ in their response to a reduction in condition, and (3) how the reduction in the extent of sexual dimorphism in response to a reduction in condition 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 suppressed to prevent the model from having traits interact with each other.

Random-effects formula:  $(\text{trait}-1|\text{units})$

By including unit as a random effect, we are estimating the mean and distribution of individual effects on each of our traits. 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. For the wing and leg model, we used the *qqnorm* and *qqline* function.

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. We did observe high collinearity of residuals. However, 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 also 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 and proceeded.

When we ran the *qqnorm* and *qqline* function to test our model for wing and leg comparisons, the line generated through the first and third quantiles remained relatively close to our data points, so we moved ahead with that model.

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

We were unable to generate treatment contrasts for sex using the model run in *lmer*. However, the model was used to generate treatment contrasts between the sex and condition levels. The contrasts generated using *emmeans* made senses, biologically given the values we inputted. The contrasts were also cross-referenced with an identical model run in *lm* (i.e., random effects were dropped – see below), and provided very similar results. However, the contrasts generated using our mixed model in *lmer* generated confidence intervals that mostly did not include zero, while our contrasts generated using our fixed effect model in *lm* all included zero, suggesting different ‘significance’ of our results (See Figures 2 and 3)

#### *Multivariate single effect linear model*

To get proper effect sizes for our variables of interest (sex and condition, and their interaction) 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 the linear model to generate coefficient plots, and treatment contrasts using *emmeans*.

For comparison between wing and leg traits, random effects were also dropped in favour of doing a simpler multivariate linear model that focused on condition and sex, as the random effects between individuals were not strong enough to be necessary for inclusion when comparing the sexually dimorphic traits to wing size.

## **Results**

Our results suggest that while depriving flies of nutrition during larval development decreases the extent of sexual dimorphism in the sexually selected forelegs, the reduction in condition does not do so to an extent that is biologically relevant. The effect of condition on all four traits is negative, indicating that our starvation treatment did reduce overall body size and foreleg size (Figure 1). Condition had a similar effect on body size and our foreleg traits, indicating that a reduction in condition did not generate disproportionate reduction in the forelegs relative to body size. Sex was the dominating effect on all traits, except thorax size, where the effect of condition was larger. The effect of the interaction between condition and sex on all four traits was negative (but nearly zero) and non-significant, suggesting that the reduction in the extent of sexual dimorphism due to condition was not biologically relevant.

Using our mixed effects linear model, we generated two-way interaction treatment contrasts between sex and condition to determine if the magnitude of the reduction in sexual size dimorphism (i.e., difference in size between males and females) across our leg traits, and

between our leg traits and body size. There was no likely no biologically relevant change in sexual dimorphism in tarsus length (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, again confirming the absence of disproportionate effect of condition on the sexually selected forelegs. Our two-way interaction contrasts using our linear model confirm the pattern observed in the previous analysis, however, all confidence cross zero, indicating 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 effect of condition on traits size in males vs females is not biologically relevant.

To determine if depriving flies of nutrition during larval development had a greater effect on the more sexually dimorphic forelegs in comparison to the wings of *D. prolongata*, we looked at the effect of sex, condition, and the interaction between condition and sex on the size of foreleg traits, body size, and wing area.

Just like the effect of condition on the leg and body traits, the effect of condition on wing area is also negative, which indicates that the starvation treatment also reduced wing area (Figure 5). Condition had a slightly larger effect on wing area than the body and foreleg traits, indicating that there was a slightly disproportionate reduction in wing size in comparison to the other traits. Sex still had a positive effect on wing size, indicating male wings are a little bit larger than females, however as expected the effect is not as large on wing size as it is on the leg traits. Unlike the leg traits, the effect of the condition was larger than the effect of sex. Similarly, to the leg traits, the effect of the interaction between condition and sex on wing area was negative and nearly zero, however in the case of wing area the confidence intervals did not overlap with zero, suggesting that wing area may have been slightly more sensitive than the sexually dimorphic traits.

Using a fixed effects linear model, we generated high condition and low condition treatment contrasts with sex to determine how the difference in trait size between males and females affected their dependence on condition. The confidence intervals for low and high concentration overlap with each other and zero, however the low and high concentration confidence intervals for wings do not cross zero or overlap (Figure 6). This suggests to us that the wing traits were actually a little bit more sensitive to starvation than the leg traits, the opposite of what we expected in our hypothesis.

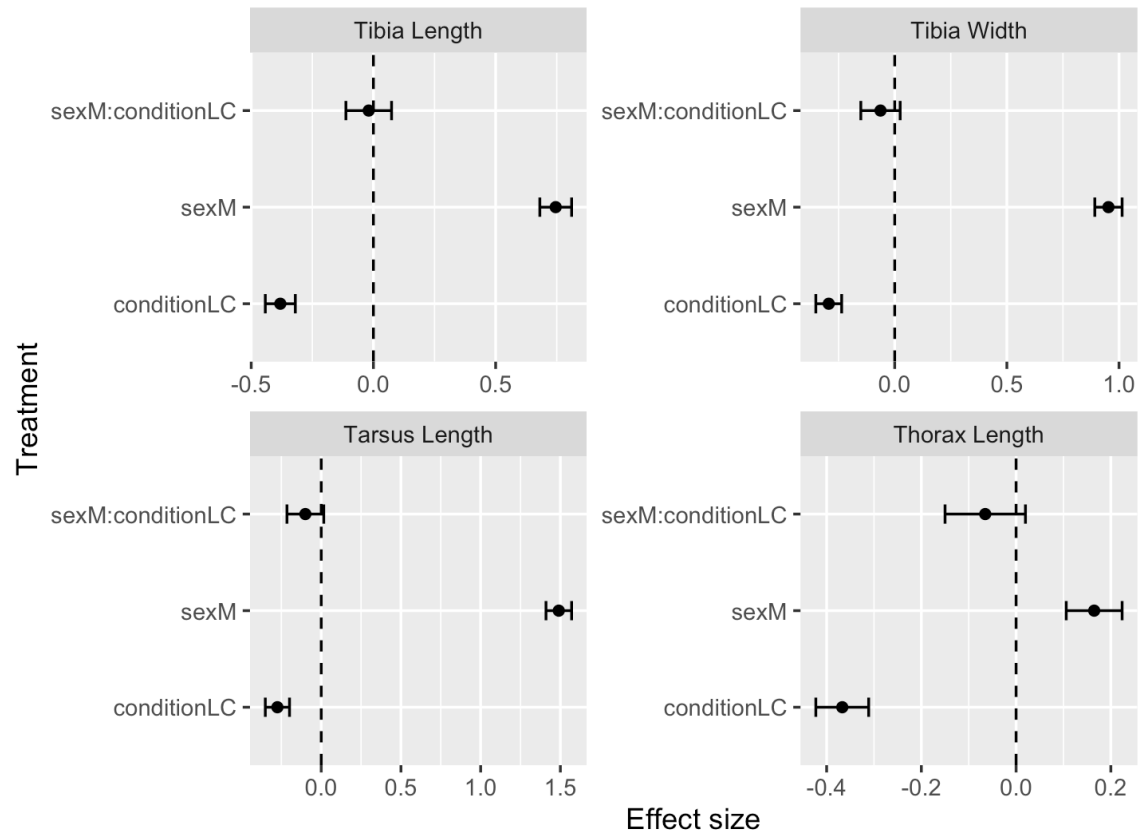


Figure 1. The effect of sex, condition, and their interaction on foreleg and body size. 95% confidence intervals are shown. All response variables have been log2 transformed.

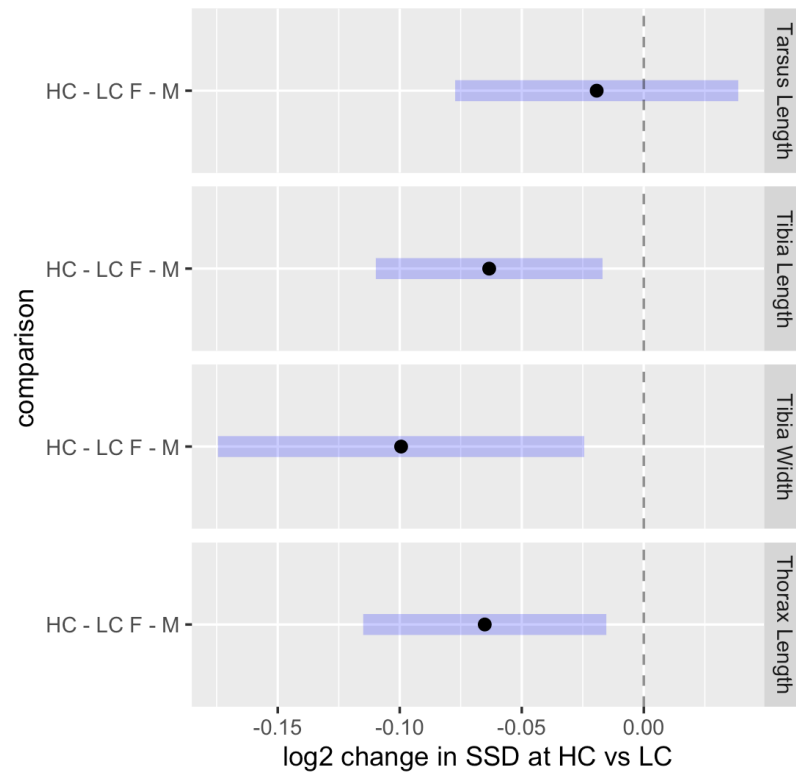


Figure 2: Log2 change in SSD at HC vs LC using the multivariate mixed model in lmer. Bars show 95% confidence intervals.

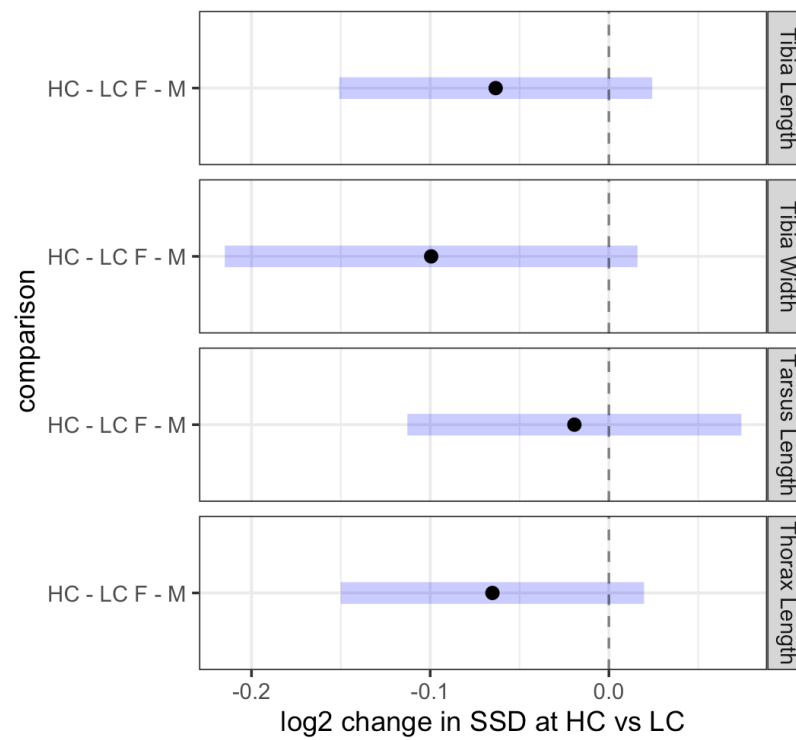


Figure 3. Two-way interaction contrasts showing Log2 change in SSD at High Condition (HC) and Low condition (LC) in 3 foreleg traits and body size. Bars show 95% confidence intervals.

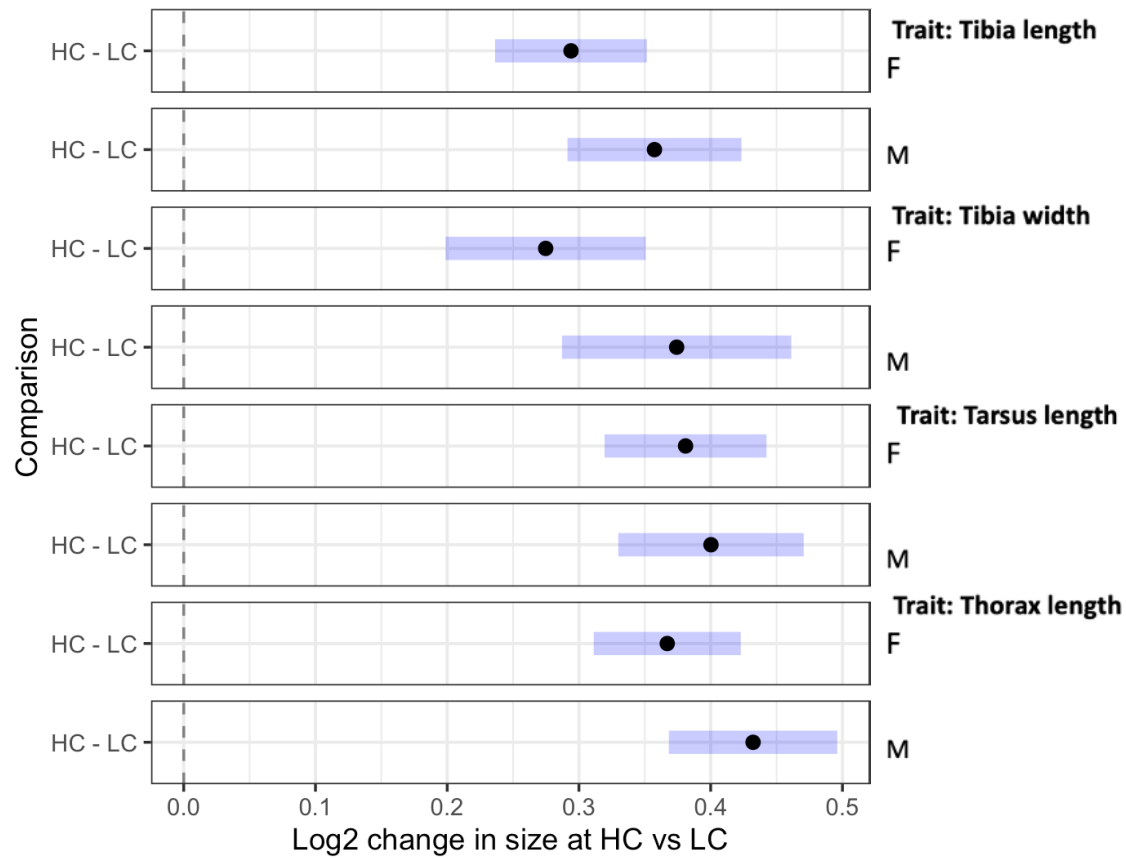


Figure 4. Log2 change in trait size for at high condition (HC) vs low condition (LC) in males (M) and females (F) in foreleg traits and body size.



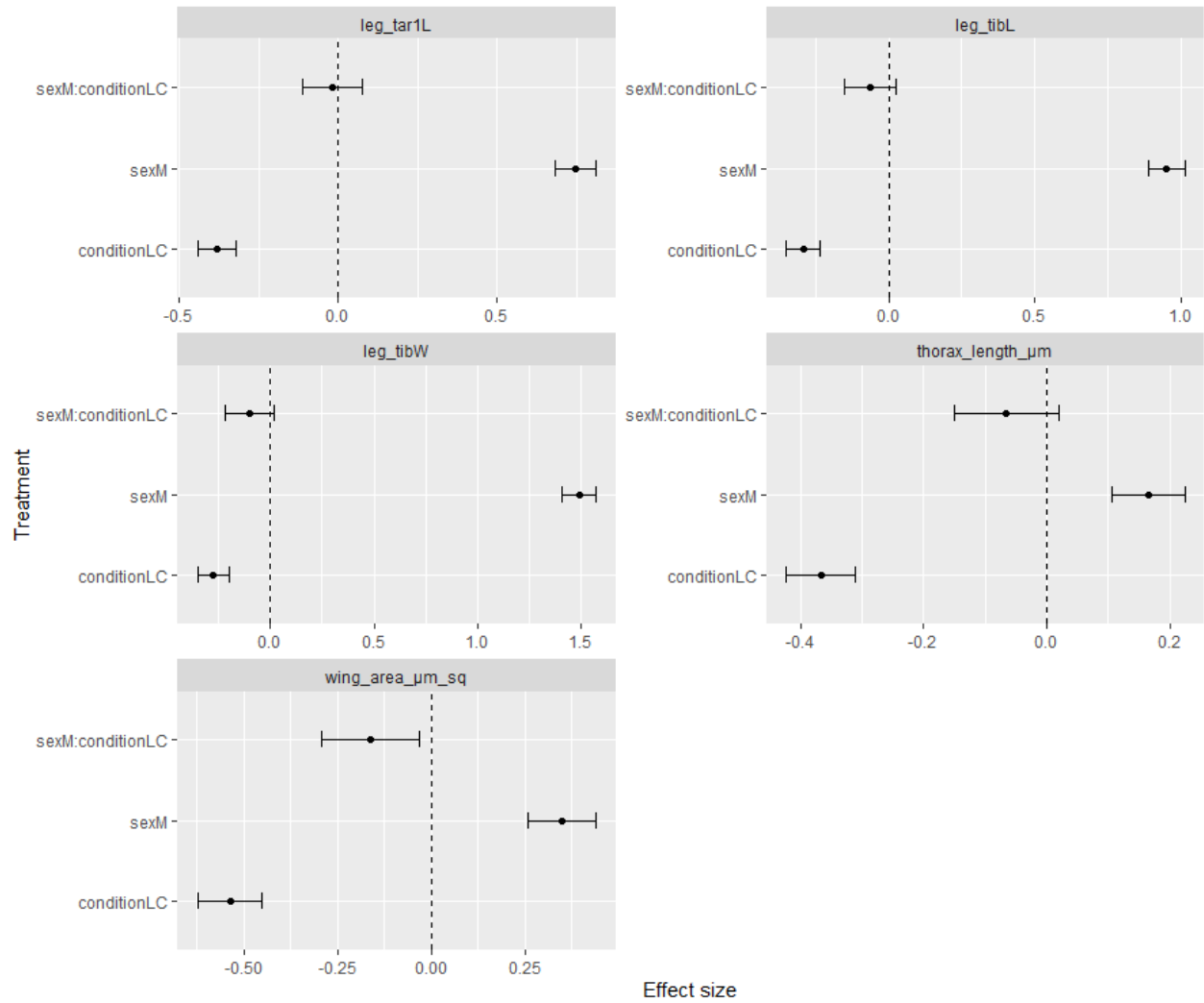


Figure 5. The effect of sex, condition, and their interaction on foreleg, body size, and wing size. 95% confidence intervals are shown. All response variables have been log2 transformed.

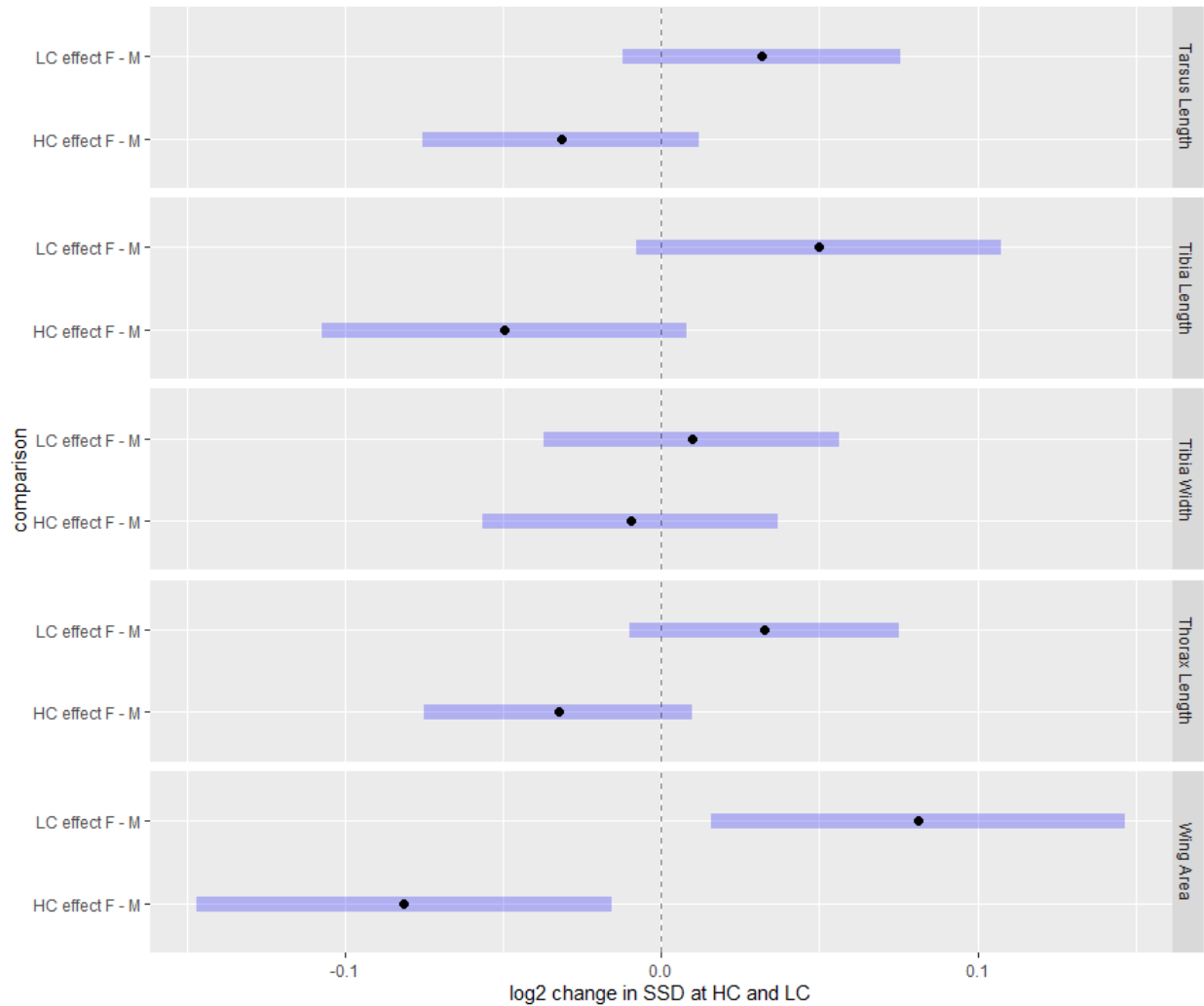


Figure 6. Log2 change in SSD at HC and LC using the multivariate fixed effects model in lm. Bars show 95% confidence intervals.

## Discussion

To summarize our results, while we were able to see that the starvation treatment resulted in a negative effect on all traits, including wing area, when looking at the interaction of condition and sex, wing area did not overlap with zero and indicated it might have been a little bit more sensitive to starvation than the leg traits. However, wing area was not different from the leg traits by a large degree, being just past zero at the outer points of its confidence intervals. In the fixed effects linear model, we can see a clearer separation between the condition effects in wing area without any overlap in confidence intervals, which again suggests to us that wing area was the trait that was most sensitive to starvation, which is the opposite to our initial hypothesis. Being able to perform further tests to see if this is a consistent result across different strains and species would be ideal, given that most results that may be biologically relevant in our wing comparison data is not a strong effect that is immediately obvious.

For the comparison of leg traits specifically, there were no distinct traits that stood out, with confidence intervals that mostly overlapped. In Figure 4, the leg trait with the highest

relative change in trait size, tarsus length, still overlapped with the trait that had the lowest relative change in trait size, tibia width in females and tibia length in males. This again tells us that while there was some consistency in the relative change in size of leg traits, it is probably not significantly related to any sexually dimorphic effects, supported by Figure 2 and 3 where only small variations were observed between treatments and intervals crossing or hovering near zero.

### *Biological interpretation*

Our results 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 biologically relevant manner. We also see a small, but not pronounced male-bias in the expression of condition-dependence in forelegs. Additionally, contrary to our hypothesis, it looks like sexual dimorphism does not increase the condition dependence of a trait, in fact wing area was more sensitive to starvation than the leg lengths and widths.

### *Future steps*

As mentioned, given our results, we think a good next step would be to perform this experiment again in *D. prolongata* under the same conditions to see if these results are replicated, since we obtained results that were not biologically significant, or in the case of the wing versus leg comparisons, very minor differences. Additionally, it may be beneficial to repeat this experiment in other species of *Drosophila* that exhibit some form of sexual dimorphism to obtain a more generalizable pool of results than can be obtained from a single species. Looking past flies and looking at other model organisms that can exhibit sexual dimorphism like butterflies would also increase confidence in results if some consistency between organisms is observed.

While our data set did not include second or third leg measurements for *D. prolongata*, we think it would be a good idea to perform a similar comparison to the one we did between wing and leg size but just between each of the three leg measurements for each leg. We suggest this because while the forelegs of *D. prolongata* are sexually dimorphic, the second and third legs are not nearly as extreme. This may provide us with a more meaningful comparison than comparing wings to legs since they are more similar to one another.

One package that we did not use in our analysis was MCMCglmm, which can be used to produce generalised linear mixed models. Since our data has many response traits that we analyzed, this package's ability to create residual and random-effect variance structures could be valuable for the future proposed analyses. We could also use this package to perform more detailed covariance analyses.

## References

- Bonduriansky, R. (2007). The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition-dependence. In *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism* (pp. 176–184). Oxford Academic.  
<https://doi.org/10.1093/acprof:oso/9780199208784.003.0020>
- Emlen, D. J. (2008). The Evolution of Animal Weapons. *Annual Review of Ecology, Evolution, and Systematics*, 39(1), 387–413. <https://doi.org/10.1146/annurev.ecolsys.39.110707.173502>
- Luecke, D. M., & Kopp, A. (2019). Sex-specific evolution of relative leg size in *Drosophila prolongata* results from changes in the intersegmental coordination of tissue growth. *Evolution*, 73(11), 2281–2294. <https://doi.org/10.1111/evo.13847>
- Pesevski, M. (2021). *Influence of environmental variation on sexual dimorphism in Drosophila morphology among adaptively diverged populations and in an inter-specific comparative context* .
- Rowe, L., & Houle, D. (1996). The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263(1375), 1415–1421. <https://doi.org/10.1098/rspb.1996.0207>
- Setoguchi, S., Takamori, H., Aotsuka, T., Sese, J., Ishikawa, Y., & Matsuo, T. (2014). Sexual dimorphism and courtship behavior in *Drosophila prolongata*. *Journal of Ethology*, 32(2), 91–102. <https://doi.org/10.1007/s10164-014-0399-z>
- Stillwell, R. C., Dworkin, I., Shingleton, A. W., & Frankino, W. A. (2011). Experimental Manipulation of Body Size to Estimate Morphological Scaling Relationships in *Drosophila*. *Journal of Visualized Experiments*, 56. <https://doi.org/10.3791/3162>

Toyoshima, N., & Matsuo, T. (2023). Fight outcome influences male mating success in *Drosophila* *prolongata*. *Journal of Ethology*, 41. <https://doi.org/10.1007/s10164-023-00778-1>