Box 1 - TDT Curves

## Estimate TDT Curve Parameters for *Drosophilia*

We can have a look at each new species’ TDT curves collected by Vanessa ([Figure 1](#fig-tdt)). We can also use the data to calculate the TDT curve (slope) and for each species (Table 1).

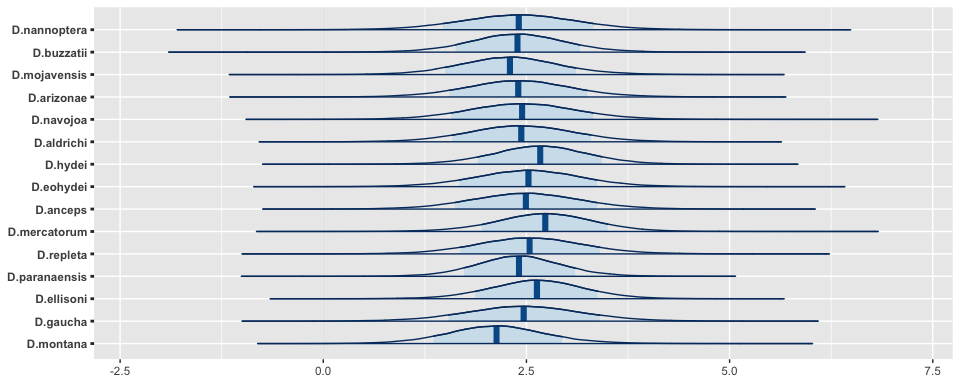
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| **Figure** 1- TDT curves for each species. The x-axis is temperature and the y-axis is the log10 of the time to coma. |

**Table** **1:** Estimated parameters for each of the 8 new species.

| **Species** | **Slope** | **CTMax** |
| --- | --- | --- |
| bip | 2.5 | 41 |
| bir | 3.1 | 42 |
| hydei | 2.9 | 42 |
| kik | 3.3 | 42 |
| ps | 2.6 | 41 |
| rub | 2.0 | 39 |
| sim | 2.5 | 41 |
| sulf | 2.0 | 39 |

## Applying Multivariate Modelling Approaches

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| **Figure** 2- Phylogenetic relationships among r length(tree$tip.label) *Drosophila* species. |



## Box 1 – Thermal Death Time framework case studies

Parameters from Thermal Death Time (TDT) curves provide a powerful tool for understanding the thermal sensitivity of organisms. Comparative approaches using such data can provide a way for us to evaluate differences in population and species sensitivity, and identify the key ecological and life-history traits that increase vulnerability to heat stress. Nonetheless, while static measures of thermal tolerance (e.g., ) are widespread, parameters from TDT curves are much less frequently estimated. In addition, we have a poor understanding of how dynamic (TDT) and static () lethal measures correlate with each other and with sub-lethal measures of damage, such as critical fertility limits (CFLs). Identifying the correlation between lethal and sub-lethal limits can provide powerful ways to further clarify species vulnerability to heat stress that might lead to population extinction well before mortality is identified.

A number of significant challenges imped are ability to take the TDT and sub-lethal frameworks into a comparative context. First, ethical, conservation, and logistical challenges limit our ability to measure and estimate key parameters for many species, creating significant gaps in our knowledge across the tree of life. Second, while there has been a focus on species-level responses, population-level information is seldom incoperated into comparative frameworks limiting our ability to decompose sources of variance1.

Powerful multivariate hierachical models (i.e., multi-response models) are capable of dealing with missing data, and provide a means by which multiple physiological thermal tolerance measures can be modelled simultaneously1,2. When used in conjunction with an understanding of the phylogenetic relationships among species and/or populations, such modelling approaches can provide informed estimates for species missing data and explicitly estimate the covariance between tolerance measures while accounting for their uncertainty. Such insight can be used to inform policy and conservation-related decision making for species with little to no data.

#### Application of Multivariate Comparative Approaches using Thermal Tolerance Measures: A case study using *Drosophilids*

We integrated static lethal limits () collected by3 and XX with dynamic lethal limit measures from TDT curces [i.e., slope () and intercept ()] estimated by XX. We also collected data to estimate TDT curve parameters for eight additonal species following protocols outlined in4. Critical fertility limits for *Drosophilids* were compiled from5 and3. These data were integrated with data on lethal limits at the species-level. Overall, we obtained data for 117 species ( = 117 species; = 51 species and = 51 species). When the same species were sampled multiple times we retained within species variation in models. We then fit the following model in R (vers. 4.2.1)6 with the package *MCMCglmm* (vers. 2.34), which uses data augmentation during model fittng to estimate missing data7:

where is the overall mean (intercept) for each trait for each observation, *i*, for species, *k*; and are phylogenetic (evolutionary) and species effects (i.e., ecological species effects) (assumed to be sampled from a normal distribution) with the evolutionary relatedness being structured according to the phylogenetic relatedness matrix () as opposed to an identity matrix (). Heat tolerance measures are modelled according to a multivariate normal distribution. Covariances between thermal tolerance measures at each random effect level are also estimated.

##### Evolutionary signatures of thermal tolerance in Drosophilids

Models including phylogeny were better supported than models without phylogeny ( = 17.94), with all traits exhibiting a moderate to strong phylogenetic signal indicating evolutionary conservatism in heat tolerance (Phylogenetic : – 0.25, 95% CI: 0.08 to 0.49; – 0.51, 95% CI: 0.23 to 0.76; CFLs – 0.53, 95% CI: 0.12 to 0.76). While the model also suggested that there was within species variation, missing data and lack of replicate species populations make conclusions premature (: – 0.09, 95% CI: 0.03 to 0.28; – 0.37, 95% CI: 0.15 to 0.67; CFLs – 0.04, 95% CI: 0.01 to 0.34).

##### Evolutionary relationships can help understand thermal tolerance in species lacking data

The model predicted known data reasonably well for most traits (correlation between known values and values estimated from the model: – 0.94, 95% CI: 0.86 to 0.98, p < 0.001; – 0.69, 95% CI: 0.58 to 0.78, p < 0.001; CFLs – 0.91, 95% CI: 0.85 to 0.95, p < 0.001), suggesting that even this basic model could be used to provide sensible quantitative measures for *Drosophila* species without heat tolerance estimates. While modelling approaches such as these are not a replacement for actual empirical data, they could be used to provide informed judgements on thermal tolerance in the absence of such information, but should be tested (using cross-validation) and refined when necessary.

##### Static lethal limits are evolutionarily correlated with sub-lethal limits in Drosophilids

Interestingly, there was a reasonably strong phylogenetic correlation between and critical fertility limits in *Drosophilids* (r = 0.75, 95% CI: 0.04 to 0.94), but not between , CFLs and the slope from TDT curves (cor(, ) = -0.07, 95% CI: -0.7 to 0.57; cor(CFLs, ) = -0.06, 95% CI: -0.71 to 0.58). These results suggest that sub-lethal measures, such as CFLs strongly coincide with a species’ upper lethal limit. It would be interesting to understand if this is a general phenomena across more taxonomic groups.

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| **Figure** 3- Species included in the dataset and their phylogenetic relationships. |

## References

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