Modelling Thermal Responses of Metabolic Traits

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Abstract

Temperature is the one of the most important factors in determining metabolic rate and the optimisation of metabolic rate will tend to increase the fitness of any organism. Climate change is already having an enourmous impact on all life on Earth and ectotherms can be particularly sensitive to environmental shifts in temperature. Many species are resorting to latitudinal range shifts, as well as behavioural and evolutionary adaptation, but extinction is often unavoidable. Improving our ability to model thermal responses is vital if we are to predict, understand, and hopefully prevent, future extinctions. This study compared the relative ability of three models, the cubic polynomial, Briere, and Schoolfield, each being fitted to 1367 datasets within the BioTraits meta-dataset. It was concluded that the Briere model was the best based on these data, affirmed by consistently better AIC_c scores.

I. Introduction

Temperature is a fundamental parameter in almost all biological processes and its importance in metabolic biology is well documented (Brown et al. 2004, Montoya et al. 2012, Dell et al. 2011, DeLong et al. 2017). Metabolic processes are often catalysed by enzymes which depend on kinetic, and ultimately heat, energy to function. As temperature decreases, molecules have less and less kinetic energy, decreasing the acquisition rate of substrate by enzyme units, and thus metabolic rates decrease accordingly. When temperature increases, metabolic rates increase steadily until the thermal optimum is reached (DeLong et al. 2017, Dell et al. 2011); that is, the temperature at which optimal metabolic rate occurs (T_{pk} or T_{opt}). Beyond this, increasing temperature will start to hinder the metabolic rate until the

lethal limit is reached. The mechanisms behind this sharp decline in metabolic rate are disputed but could potentially be due to protein degradation (Johnson & Lewin 1946, Dell et al. 2011). These metabolic responses to temperature exhibit a remarkably similar pattern graphically, often referred to as Thermal Performance Curves (TPCs), across a plethora of metabolic processes and taxa. This makes the study of TPCs a useful tool of comparison for all life on Earth as all living things depend on metabolism for their energy. Furthermore, an increased understanding of how species respond to temperature is imperative in a rapidly warming world. If we can find some plasticity in a species' temperature tolerance then perhaps it will have a better chance of avoiding the mass extinction that is sweeping our planet, although some recent findings suggest the scope for adaptation may be limited (Tüzün & Stoks 2018). Of course, there are other ways a species may adapt, for example through latitudinal range shifting (Haase et al. 2019) or evolution. However, the latter seems unlikely in the time-frame available, although some studies have suggested it is possible (DeLong et al. 2018), and the former is only possible for mobile species with suitable habitats to move to. The database used in this study, BioTraits, was provided by my supervisor, Dr. Samraat Pawar, and is an extension of the database used by Dell et al. (2011) and Kontopoulos et al. (2018). It is one of the most extensive metabolic datasets ever amalgamated and provided an excellent way to compare three different models for TPCs.

i. Models

Three models were used in this study to compare their ability to fit to each dataset within BioTraits. Firstly, there is the phe-

nomenological cubic polynomial:

$$B = B_0 + B_1 T + B_2 T^2 + B_3 T^3 \qquad (1)$$

Where B is the responding trait value, T is the temperature, B_0 is the y-intercept of the curve, and B_1, B_2, and B_3 are coefficients acting on T.

Secondly, the Briere model (Briere et al. 1999) was used as an alternative phenomenological model:

$$B = B_0 T (T - T_0) \sqrt{T_m - T} \qquad (2)$$

Where T_0 and T_m are the minimum and maximum thermal tolerances respectively for the trait - B - and B_0 is a normalisation constant. Whilst this model is technically phenomenological, it can still provide useful biological insight once fitted as it gives an estimate of the thermal tolerance of a particular trait for a particular organism; as it has x-intercepts (T), and its parameters meet the definition of 'ecological parameters' coined by (Lamb et al. 1984). However, it falls short of the definition of a mechanistic model as the model provides no insight into the underlying biological mechanisms at work.

Finally, the third model used in this study was a simplified version of the Sharpe-Schoolfield (Schoolfield et al. 1981) model to provide a mechanistic comparison as it is derived from thermodynamic and enzyme kinetic theory. The full model is given by:

$$B = \frac{B_0 e^{\frac{-E}{k}(\frac{1}{T} - \frac{1}{283.15})}}{1 + e^{\frac{E_l}{k}(\frac{1}{T_l} - \frac{1}{T})} + e^{\frac{E_h}{k}(\frac{1}{T_h} - \frac{1}{T})}} \qquad (3)$$

Where k is the Boltzmann constant (8.617×10^{-5} eV $\dot{K}^{-1}$, Boltzmann (1872)), T is the temperature in Kelvin, B_0 is the trait value at a reference temperature - 283.15 K in this study - E_l is the low-temperature deactivation energy (eV) of the enzyme and controls the behaviour of the curve at very low temperatures and T_l is the temperature at which 50% of enzyme units have been low-temperature deactivated. E_h is the high-temperature deactivation energy of

the enzyme and controls the behaviour of the curve beyond T_{pk}, and T_h is the temperature at which 50% of enzyme units have been high-temperature deactivated. E is the activation energy which controls the behaviour of the curve in the enzyme's 'normal operating range', that is, before T_{pk} but not at low temperatures. This model attempts to capture the non-linear rate acceleration (E_l) at low temperatures, the linear rate increase, E, up to T_pk, and rapid non-linear deceleration, E_h, at high temperatures as the temperature approaches the lethal limit of an enzyme. This makes the Briere model an interesting comparison as this too attempts to capture the two non-linear and linear aspects of a typical TPC. There are also two simplified versions of the Schoolfield model, each with either the E_h or E_l element removed from the denominator. The removal of E_l and associated terms is sometimes intuitive as low-temperature deactivation of enzymes is weak and there is often insufficient data at low temperatures, as is the case with BioTraits. For these reasons, this simplified version was used for this study and is given by:

$$B = \frac{B_0 e^{\frac{-E}{k}(\frac{1}{T} - \frac{1}{283.15})}}{1 + e^{\frac{E_h}{k}(\frac{1}{T_h} - \frac{1}{T})}} \quad (4)$$

Simplifying the model also allows for more datasets to be explained as the minimum number of datapoints required for the six-parameter model would be larger than for the four-parameter simplified version. Other studies, such as Alber & Schaffner (1993), have demonstrated that the simplified Schoolfield models often fit better. The reference temperature for this study was set at 283.15 K as it has proved a reliable reference in other studies of this nature (Dell et al. 2011) and seems more appropriate for the many cold-adapted species represented in BioTraits, despite Schoolfield et al. (1981) using a reference of 298.15 K. Assuming there has been no low- or high-temperature

deactivation B_0 can be used as an approximation of the trait value at this reference temperature. This study will be based on comparisons of these models, in the vein of Johnson & Omland (2004), as opposed to traditional null-hypothesis testing, as they demonstrated the power of model comparison with large sets of observational data.

II. Methods

i. Computing Languages

R

R (3.2.3) is a popular programming language within the biological sciences and is particularly powerful for statistical analysis, data manipulation, and data visualisation (R Core Team 2015). RStudio (1.1.419) was used as an interactive development environment (IDE) for R in this study as it permits the display of graphics and code within the same pane which is useful for visualisation (RStudio Team 2016). R was predominantly used in this study to 'wrangle' BioTraits, that is, to prepare a filtered version for model fitting. It was then used post-fitting to visualise the models with optimised parameters, in particular making use of the package ggplot2 (3.1.0) (Wickham 2016) to create aesthetic and intuitive plots.

Python

Python (3.5.2) is an immensely popular, object-oriented programming language first developed by van Rossum (1991). It can lend its popularity, in part, to its extensive collection of packages, as well as its intuitive syntax, making it one of the most versatile programming langauges. In particular, the package lmfit (Newville 2014) has robust functions for optimising model parameters using non-linear least-squares techniques which were used to fit the models in this study. Furthermore, Python is relatively fast, computationally speaking, at

looping, which gives it further advantage over R for fitting models to large datasets like BioTraits. Visual Studio Code (Microsoft 2019) was used as an IDE for Python and IPython (P'erez & Granger 2007) was used as a command shell for testing code as it has particularly good debugging features.

Bash

Bash (4.3.48) is an open-source, UNIX shell and command language originally created by Fox (1989). It is the default shell language used for most Linux distributions such as Ubuntu 16.04 which was the operating system used in this study. A Bash script was used to run each of the scripts used in this project as it is a versatile language that can easily call and run other languages.

ii. Data

BioTraits consists of 2165 unique thermal responses of metabolic processes from 1010 publications. Predominantly, respiration, growth, and photosynthetic rate are the metabolic process being measured against temperature. BioTraits includes species from many Phyla with diverse life histories, but a majority of representatives are terrestrial species, Arthropods being particularly well-represented. As this dataset contains 155 columns and 25826 rows, it was first refined to a handful of columns relevant to this study to improve computational speed, namely the trait value and temperature. Rows with missing values for these columns were removed and any dataset with less than six unique datapoints was also removed, as this is the minimum required to estimate four parameter models like the cubic polynomial and simplified Schoolfield.

iii. Parameter estimation

Using R, starting parameters for every dataset in BioTraits were calculated. For the cubic polynomial model, starting values of 1 were used for all four parameters. For

Briere, estimates for T_0 and T_m were made using the minimum and maximum observed temperatures respectively. For Schoolfield, a reference temperature of 283.15 K was used and the parameter estimations were carried out following the method of Schoolfield et al. (1981). B_0 was estimated as the recorded trait value nearest to this temperature, as defined by the equation. The peak metabolic rate B_{max} was then calculated (T_{pk} being the corresponding temperature at this trait value) and the dataset was split either side of this value. If B_{max} occurred at the highest recorded temperature (i.e. the rate had not started descending yet) the dataset was not split and the subsequent regression was carried out on the whole dataset. The trait values were plotted on an Arrhenius plot ($log(trait)$ against $\frac{1}{kT}$) with k being the Boltzmann constant. Linear regression was carried out on the left-hand (below T_{pk}) data and the estimate for E was taken as the gradient of this line, with the Eh estimate being twice this value. If regression failed, default estimates of 0.65 for E and 1.3 for Eh were used as recommended defaults from the literature, and E was given bounds of 0 to 3, while Eh was bound between 0 and 6 to maintain theoretical accuracy (Montoya et al. 2012, Dell et al. 2011, Allen et al. 2006). Th was estimated by calculating the nearest recorded temperature to $\frac{B_{max}}{2}$ as Th is the temperature at which half the enzyme units have been made inactive, so this provides a good estimate, with a lower bound of T_{pk} applied (Kontopoulos et al. 2018). For datasets with no datapoints after B_{max}, Th was given a starting value equal to T_{max}.

iv. Model comparison

The Akaike Information Criterion (AIC) (Akaike 1974) was used to compare model fits within each dataset and is given by the formula, assuming the model is univariate,

is linear in its parameters, and has normally-distributed residuals:

$$AIC = 2k - 2\ln(\hat{L}) \tag{5}$$

Where $\hat{L}$ is the maximum likelihood estimation of the model and k is the number of parameters in the model. AIC rewards the relative goodness of fit between models applied to the same data but penalises number of parameters used as this can sometimes lead to overfitting. This method was chosen over similar methods such as the Bayesian Information Criterion (BIC, Schwarz (1978)) as it can be modified for small datasets and has higher success rates in picking the best model if the 'true' model is not present in a sample of models (Vrieze 2012). However, despite the existing penalty, AIC can still be prone to favouring models with more parameters if the sample size is small (benchmark of at least 40 datapoints per model parameter suggested by Johnson & Omland (2004)) and BioTraits includes several datasets that would be susceptible to this. This can be circumvented by using AIC_c (Hurvich & Tsai 1989), an extension of AIC with the addition of a further parameter penalty given by:

$$AIC_c = AIC + \frac{2k^2 + 2k}{n - k - 1} \tag{6}$$

Where n is the sample size and k is the number of parameters as before. It should be noted that as $n \to \infty$, $\frac{2k^2+2k}{n-k-1} \to 0$ and thus $AIC_c \to AIC$, making it suitable for large samples too. As some of the datasets used in this analysis had only the minimum permittable number of datapoints to fit a four- parameter model, AIC_c was used to compare models instead of AIC.

In addition to AIC_c, adjusted R^2, or $\bar{R}^2$, was used as an alternative comparison tool. Generally attributed to Wright (1921), R^2 is purely a measure of goodness of fit and is given by:

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \tag{7}$$

Where SS_{res} is the sum of the squared residuals between the model and observed data and SS_{tot} is the total sum of squares. A score of 1 is a 'perfect' fit and a negative score is considered a worse fit than a straight, horizontal line through the observed mean. R^2 is very susceptible to overfitting as the addition of a new parameter will always improve the score and this can lead to poor parameter estimatation which is particularly bad for model comparison (Johnson & Omland 2004). Fortunately, this can be accounted for to an extent with adjusted R^2, $\bar{R}^2$:

$$\bar{R}^2 = 1 - (1 - R^2)\frac{n-1}{n-k-1} \qquad (8)$$

Where n is the number of datapoints and k is the number of parameters. Unlike R^2, it only improves its score if an additional parameter improves the model more than would be expected by chance, making it less susceptible to overfitting and a better comparative tool for this study.

III. Results

Of the 2165 datasets in BioTraits, 1367 remained after the vetting criteria was implemented. Starting parameters were then calculated for each dataset in R with varying success. These parameters were then optimised in Python with no failed convergences (Table 1). For Briere, the interquartile ranges of B_0, T_0 and T_m were fairly narrow, as were the converged parameters for Schoolfield, although these were subject to

Table 1: *Median parameter estimates after optimisation*

	Cubic	Briere	Schoolfield
B_0	2340	0.0001434	1.271
B_1	-23.62	-	-
B_2	0.08193	-	-
B_3	-9.163e-05	-	-
T_0	-	277	-
T_m	-	318.5	-
E	-	-	0.6356
E_h	-	-	1.558
T_h	-	-	303.2

far stricter bounds. The datasets and associated optimised parameters were returned to R for visualisation and comparitive statistical analysis. An example plot is shown in Figure 1 and summarised AIC_c and $\bar{R}^2$, values are shown in Table 2. Mean $\bar{R}^2$ indicated that the cubic model had the best average fit, with Schoolfield second and Briere last. Interestingly, Table 2 shows that despite this, if the best $\bar{R}^2$ counts are compared, that is, the number of times each model had the highest R^2 value rather than the average, cubic is still the best, but Briere replaces Schoolfield as second best. With regards to AIC_c, Briere is considered the best model in the highest number of datasets, with cubic second best and Schoolfield worst.

Table 2: *Summary of comparative statistics for the three models. The first and second rows are counts and the third row is the median adjusted R^2*

	Cubic	Briere	Schoolfield
Best AIC_c	372	549	203
Best adjusted R^2	708	336	306
Average adjusted R^2	0.8507	0.6655	0.7343

IV. Discussion

For Schoolfield, the regression carried out on the Arrenhius-transformed data was unreliable at providing 'reasonable' starting estimates and defaults were often used as a result, as this provided better fits in those cases. 'Reasonable' estimates were defined as within or near the range established by Dell et al. (2011); for E this was $0.2 - 1.2$. This may have been because a lot of the datasets used were quite small, and, after being split about B_{max}, could lead to unrepresentative regression on the Arrhenius plots, leading to poor estimates for E and E_h. However, the apparent weakness in starting parameter estimation seemed to be mitigated by the use of literature defaults as the average optimised parameters were similar to other studies (Dell et al. 2011, Kon-

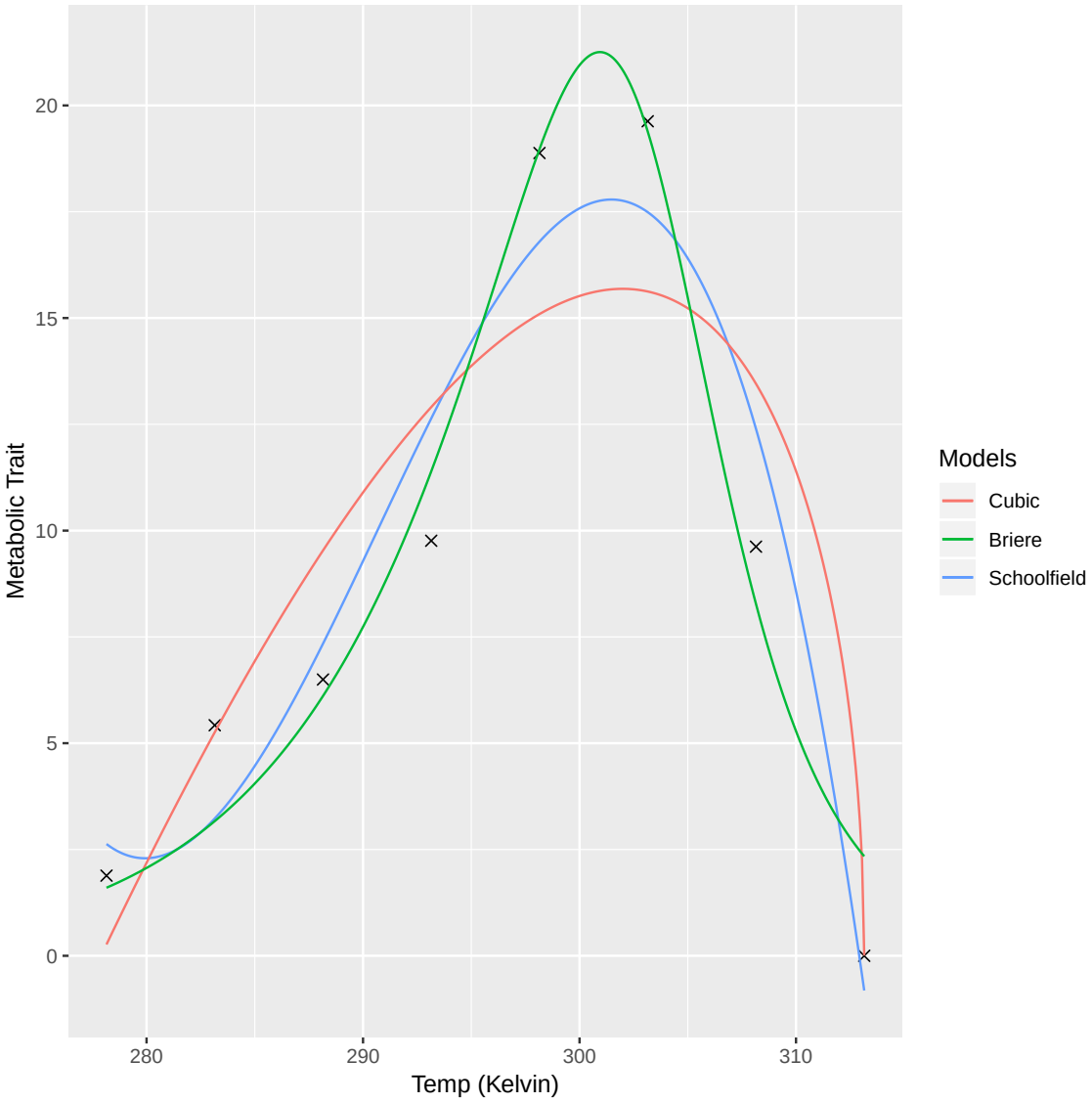

Figure 1: *An example plot of the three models fitted to data used by (Li et al. 2009)*

topoulos et al. 2018). Average optimised parameters for T_0 and T_m in the Briere model were slightly lower and higher respectively than the estimates of (Briere et al. 1999) ($T_0 = 283.15K$, $T_m = 310.15K$). This may be due to the larger and more taxonomically diverse nature of BioTraits as Briere's study was concerned exclusively with Arthropods.

Comparing the models produced some conflicting but altogether more interesting results. Cubic was the best fit according to the average $\bar{R}^2$ but this is not particularly surprising when one considers the freedom with which its parameters can be varied without theoretical and biological bounds. Of the other two models, Schoolfield had the higher average $\bar{R}^2$ but Briere had a higher $\bar{R}^2$ in more datasets when count was considered, implying Briere had a greater range of fit consistency. As the Briere model is based predominantly on the thermal responses of Arthropod metabolism, perhaps for these species it fit well and for other Phyla it was less competent. Conversely, comparison through AIC_c showed Briere to be the best model in the highest number of datasets, with cubic second and Schoolfield third. The discrepency between the two comparative measures is intriguing and may be due to the additional parameter penalty that AIC_c incorporates which would favour the Briere model over the other two as it requires one fewer parameter. Moving forward, further research in this field would benefit by comparing more models, such as those proposed by DeLong et al. (2017), and more data will undoubtedly improve reliability. Whilst BioTraits is one of the most extensive datasets of its kind, it still shows clear bias towards certain Phyla, life histories, and climates, which will hinder any extrapolations that can be drawn from models it fits well to.

In conclusion, the cubic model did fit well on average and was well-supported by

$\bar{R}^2$, but the application of a purely phenomenological linear model to the proven non-linear response of metabolism to temperatures would limit any useful biological interpretation and prediction going forward. The Schoolfield model has the advantage of parameters based on biological mechanisms but this study showed it was significantly less able to explain the data, particularly when AIC_c was considered. Briere, however, proved to be the best model, at least for BioTraits. It also has a distinct advantage over the cubic model in that, despite its lack of mechanistic-underpinning, useful biological inferrences can be made when it is fit well.

Ultimately, the 'best' model depends on the question you are asking. If the understanding of mechanism is your goal, Schoolfield is still the best, as it is the only one of these three that is based on such theory. If you are interested in thermal tolerances, then the Briere model is a clear winner and if you wish only to identify an average response for a particular organism or species then the cubic model is still a useful tool.

Levins (1966) said that the unavoidable paradox of model fitting in biology is that one must sacrifice either generality, precision, or realism in the formulation of biological models. Whilst many may dismiss the cubic model for its lack of mechanistic property, is sacrificing realism in using a phenomenological model like the cubic worse than sacrificing precision by using a mechanistic model like Schoolfield? I would argue it is not.

Mention affect of bad B0 for schoolfield Akaike weights and tings Growth rate tends to have a non-linear relationship for arthropods (Briere et al. 1999) which may make the cubic model less useful for interpretation. The Briere curve intercepts the tem-

perature axis at high and low temperatures, allowing estimation of an upper and lower trait threshold. AICc a much better comparison tool than $\bar{R}^2$ (Johnson & Omland 2004) The cubic polynomial may have no biological underpinning but is sacrificing realism much different to sacrificing precision (Levins 1966)?

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