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Keywords

activation energy; protist; thermal adaptation; thermal diversity; warming

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NOTE

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ABSTRACT: Conventional analyses suggest that the metabolism of heterotrophs is thermally more sensitive than that of autotrophs, implying that warming leads to pronounced trophodynamic imbalances. However, these analyses inappropriately combine within- and across-taxa trends. Our new analysis separates these, revealing that 92% of the difference in the apparent thermal sensitivity between autotrophic and heterotrophic protists does indeed arise from within-taxa responses. Fitness differences among taxa adapted to different temperature regimes only partially compensate for the positive biochemical relationship between temperature and growth rate within taxa, supporting the hotter-is-partially-better hypothesis. Our work highlights the importance of separating within- and across-taxa responses when comparing temperature sensitivities between groups, which is relevant to how trophic imbalances and carbon fluxes respond to warming.

Keywords: activation energy, protist, thermal adaptation, thermal diversity, warming.

Introduction

If temperature rises by 30°C, heterotrophic respiration rates are argued to increase 16-fold while photosynthetic rates increase only fourfold (Allen et al. 2005), illustrating that heterotrophic metabolism increases more rapidly than photosynthesis, leading to trophic and ecosystem imbalances. This widely accepted adjunct of the metabolic theory of

ecology drives current perceptions of how terrestrial and aquatic ecosystems will respond to temperature changes (Allen et al. 2005; López-Urrutia et al. 2006). Warming-enhanced respiration compared with photosynthesis may increase atmospheric CO₂ and reduce the ability of ecosystems to sequester carbon, providing positive feedback to climate change (Laws et al. 2000; Allen et al. 2005; López-Urrutia et al. 2006; Wohlers et al. 2009; Cael and Follows 2016). Such predictions rely on the assumption that auto- and heterotrophic rates differ substantially in their thermal sensitivities. Accurate estimates of thermal sensitivity of both trophic groups are therefore essential.

Both adaptive differences among taxa and thermal sensitivities within taxa can contribute to observed relationships between temperature and growth rates. On the one hand, the hotter-is-better hypothesis states that the maximal growth rates of different taxa increase with their optimal temperatures (Angilletta et al. 2010). This increasing rate of maximal growth rates with optimal temperatures across taxa is similar to the rate of how growth rates increase with temperatures within taxa. By contrast, the hotter-is-not-better (or biochemical adaptation) hypothesis suggests that the maximal growth rates are similar for taxa that have adapted to different temperatures via adjustments in physiology (fig. 1). The hotter-is-partially-better hypothesis lies between the two extremes: maximal growth rates still increase with optimal temperatures, but not as fast as the trend within taxa (Smith et al. 2019; Chen 2022; Liu et al.

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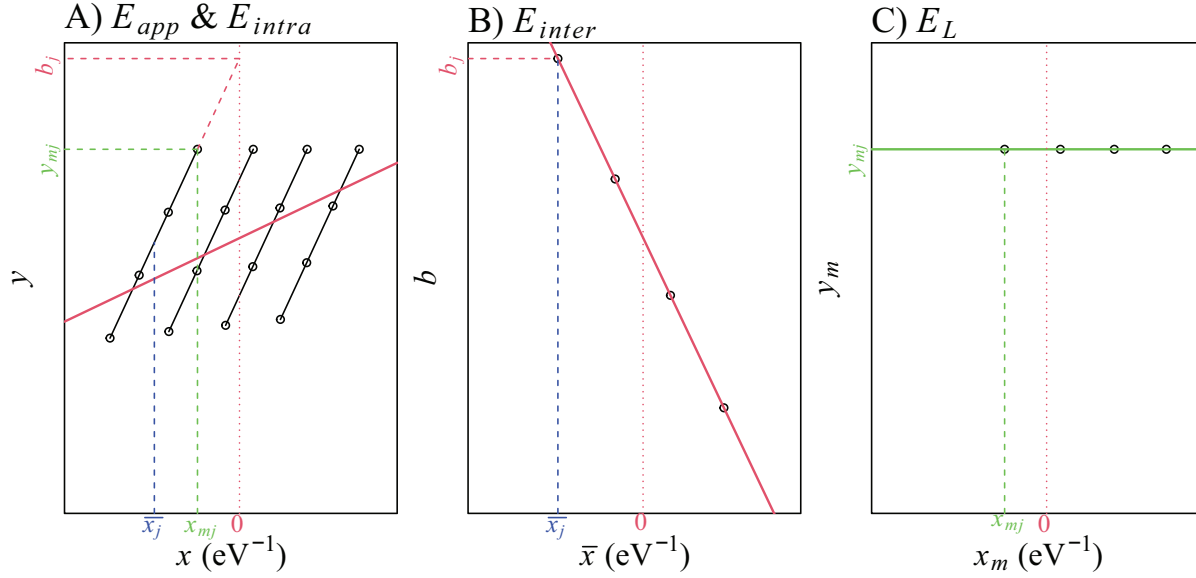


Figure 1: Relationship between apparent (E_{app} , eV), intraspecific (E_{intra}), interspecific (E_{inter}), and long-term activation (E_L) energy in an idealized case of perfect thermal adaptation. In A, black lines represent the relationship between $\ln(\text{growth rate})$ (y) and Boltzmann temperature (x) for each taxon, and their slopes are E_{intra} . E_{app} is calculated as the slope of the OLS regression (red) of all measurements. B, E_{inter} as the slope of the red regression line of the intercepts (b) of the taxa, calculated from each black regression line in A, against their mean temperatures (\bar{x}). C, E_L as the slope of the green regression line of maximal growth rate (y_m) against optimal temperatures (x_m).

2022; for a graphical illustration of all three hypotheses, see Smith et al. 2019).

Previous arguments that autotrophs are less thermally sensitive are often based on analyses that confound differences in temperature sensitivities across taxa (interspecific trend) and within taxa (intraspecific trend; note that although intraspecific differences sometimes mean genetic differences among populations, here the intraspecific temperature sensitivity is a concept of physiological response within a single population; Allen et al. 2005; López-Urrutia et al. 2006; Rose and Caron 2007; Chen et al. 2012). It is still unclear whether it is the difference in the interspecific trend or the intraspecific one that leads to the perceived difference in thermal sensitivity between autotrophs and heterotrophs. We address this issue by developing a mathematical framework that separates within- and across-taxa thermal sensitivities. We apply this approach to the per capita growth rate defined as $\mu = dN/(Ndt)$, where N is population abundance. This is a fitness metric representing the sum of metabolic activities (Savage et al. 2004). We focus on comparing autotrophic and heterotrophic unicellular eukaryotes (protists) in this study for three reasons. First, by doing so we remove confounding factors associated with multicellularity (e.g., complex life cycles). Second, protists capture much of the phylogenetic diversity of the extant biota (Caron et al. 2012; Worden et al. 2015), providing good models for metazoa and metaphyta. Finally, protists are key

players in the functioning of a wide range of aquatic ecosystems (Montagnes et al. 2008; Tréguer et al. 2018), making our analysis relevant to biogeochemical models related to climate change (Crichton et al. 2021).

By applying this new approach, we find that (1) autotrophic protists do in fact exhibit lower within-taxa thermal sensitivities than heterotrophic ones, which may relate to photosynthetic biochemistry that is temperature independent (Raven and Geider 1988; Davison 1991; Allen et al. 2005), and (2) interspecific contributions to apparent thermal sensitivities are similar between trophic groups and are less important than intraspecific contributions, supporting the hotter-is-partially-better hypothesis. Below, before presenting our analysis, we provide the background for understanding how we arrived at these conclusions.

Partitioning Activation Energies

Over a defined temperature range, growth rates are expected to increase exponentially with temperature, following the Arrhenius function ($e^{-E/(k_b T)}$, where E is activation energy, k_b is the Boltzmann constant, and T is temperature; Savage et al. 2004). A common method for assessing differences in thermal sensitivity between auto- and heterotrophs is to estimate an apparent activation energy (E_{app}) across multiple rate measurements from many species (table 1). This approach requires only individual

Table 1: Summary of regression models used in this study

Description	Model equation	Symbol definition
OLSR on the pooled data set of ln(growth rate) against temperature to estimate E_{app}	$y_i = E_{\text{app}}x_i + y_r + \varepsilon_i$	y_i : ln(growth rate of measurement i in the pooled data set) E_{app} : apparent activation energy (eV) x_i : Boltzmann temperature (eV^{-1}) of measurement i y_r : regression intercept ε_i : residual of measurement i
OLSR on the pooled data set of ln(growth rate) against temperature and ln(cell volume) to estimate E_{app}	$y_i = E_{\text{app}}x_i + \alpha_v \ln V_i + y_r + \varepsilon_i$	V_i : cell volume (μm^3) of measurement i in the pooled data set α_v : size-scaling coefficient Others are the same as above
OLSR on the data of ln(growth rate) against temperature of each taxon to estimate E_{intra}	$y_{ij} = E_{\text{intra},j}x_{ij} + b_j + \varepsilon_{ij}$	x_{ij} : Boltzmann temperature i of taxon j y_{ij} : ln(growth rate of taxon j at temperature i) $E_{\text{intra},j}$: intraspecific activation energy (eV) of taxon j b_j : normalized ln(growth rate of taxon j) [regression intercept] ε_{ij} : residual of taxon j at temperature i
OLSR of ln(normalized growth rate) against average temperature (\bar{x}) weighted by the number of observations in each taxon to estimate E_{inter}	$b_j = E_{\text{inter}}\bar{x}_j + b_0 + \beta_j$ $\frac{m_j}{M}\beta_j \sim N(0, \sigma_\beta^2)$	E_{inter} : interspecific activation energy (eV) \bar{x}_j : average temperature of taxon j b_0 : regression intercept β_j : residual of taxon j m_j : number of observations of taxon j M : total number of observations in the pooled data set σ_β^2 : variance of $(m_j/M)\beta_j$
OLSR of ln(maximal growth rate) against optimal temperature (x_m) weighted by the number of observations in each taxon to estimate E_L	$y_{mj} = E_L x_{mj} + B_0 + v_j$ $\frac{m_j}{M}v_j \sim N(0, \sigma_v^2)$	E_L : long-term activation energy (eV) x_{mj} : maximal temperature of taxon j B_0 : regression intercept v_j : residual of taxon j σ_v^2 : variance of $(m_j/M)v_j$

Note: OLSR = ordinary least squares regression.

rate measurements at any temperature. Consequently, large data sets can be compiled from the literature, since any measured rates at a known temperature may be included. The term E_{app} is then assessed by ordinary least squares regression (OLSR; table 1; fig. 1A, red line; Allen et al. 2005; López-Urrutia et al. 2006). However, this analysis conflates two processes: within-taxon (intraspecific) physiological responses and across-taxon (interspecific) thermal adaptation. It also violates the assumption that the residuals are independent (Faraway 2004), potentially leading to underestimates of E_{app} (Chen and Laws 2017).

Determining intraspecific thermal sensitivities (E_{intra}) can be achieved through controlled studies, measuring rates of a single taxon at predesigned temperatures and fitting response through the rising part of the thermal performance curves (TPCs) for individual taxa (fig. 1A,

black lines; Chen and Laws 2017; Wang et al. 2019). We can thus obtain a distribution of E_{intra} of multiple taxa, but data for this approach are sparser because of the greater effort required in obtaining TPCs than assembling single-temperature response data. A number of previous studies have also estimated E_{intra} for a number taxa from their full TPCs and evaluated differences in E_{intra} between autotrophs and heterotrophs (Dell et al. 2011; Rezende and Bozinovic 2019). However, we are not aware of any studies that partitioned E_{app} into intraspecific and interspecific components.

For interspecific thermal sensitivity, consider a case where taxa from cold environments have adapted to perform well when temperatures are low, exhibiting rates equal (or close) to those of their warmer counterparts, rather than being metabolically suppressed by cold temperatures

(Angilletta et al. 2010). Alternatively, the growth rates of taxa from warm environments may be constrained at high temperature (Flynn and Raven 2017). In either case, E_{app} estimated by OLSR will provide lower estimates than within-taxa estimates E_{intra} (fig. 1A, black vs. red lines). Here, we assess the extent to which this may occur. Although it appears straightforward to assess the extent to which E_{intra} and interspecific activation energy (E_{inter}) contribute to E_{app} , it is not a simple additive analysis (e.g., $E_{app} = E_{intra} - E_{inter}$) because of the inherent variance and stochasticity, as described below.

We derive that E_{app} can be partitioned into two components: intraspecific (within taxa; E_{intra}) and interspecific (E_{inter}) activation energy (for derivation, see supplement 1):

$$E_{app} \approx \langle E_{intra} \rangle + E_{inter} \frac{\text{Var}(\bar{x})}{\text{Var}(x)}, \quad (1)$$

where $\langle E_{intra} \rangle$ is the variance-weighted mean E_{intra} (table 2), x is the transformed environmental temperature (see “Methods”), $\text{Var}(x)$ is the variance of all x in the data set, $\text{Var}(\bar{x})$ is the variance of mean x of each taxon, and E_{inter} is derived from the OLSR slope of $\ln(\text{normalization rate})$ (the intercept of the linear regression equation used to estimate E_{intra} of each taxon) against \bar{x} . Note that both $\text{Var}(\bar{x})$ and E_{inter} need to be weighted by the number of measurements of each taxon (supplement 1). The term $\text{Var}(\bar{x})$ can be interpreted as thermal diversity, defined as the variation in organisms’ thermal preferences, as it is similar to the variance of their optimal temperatures ($\text{Var}(x_m)$; table 2). The term $\text{Var}(x)$ is the variance of the measurement

temperatures. Therefore, the contribution of E_{inter} to E_{app} is weighted by $\text{Var}(\bar{x})/\text{Var}(x)$, the ratio of taxa’s thermal diversity to the variability of the measurement temperatures. Either a weak adaptation (a small absolute value of E_{inter}) or a low thermal diversity (compared with the environmental temperature variability; $\text{Var}(\bar{x})/\text{Var}(x)$) would make E_{app} approach E_{intra} . Biologically, this means that if the taxa come from similar temperature regimes with similar adaptations to those temperature regimes, then all of the variation in apparent thermal sensitivity should be entirely due to physiological responses within taxa.

The literatures of the hotter-is-better hypothesis often focus on the relationship between maximal growth rate and optimal temperature (Angilletta et al. 2010; Smith et al. 2019; Kontopoulos et al. 2020). If we use E_L to denote the slope of the linear regression of $\ln(\text{maximal growth rate})$ and transformed optimal temperature (x_m) following Smith et al. (2019), we derive the relationships between E_{app} and E_L and between E_L and E_{inter} (supplement 1):

$$E_{app} \approx \langle E_{intra} \rangle + E_L \frac{\text{Cov}(x_m, \bar{x})}{\text{Var}(x)} - \frac{\text{Cov}(E_{intra}x_m, \bar{x})}{\text{Var}(x)}, \quad (2)$$

$$E_L \approx \frac{E_{inter} \text{Var}(\bar{x}) + \text{Cov}(E_{intra}x_m, \bar{x})}{\text{Cov}(x_m, \bar{x})}, \quad (3)$$

where Cov indicates the covariance operator.

Equations (1) and (2) offer different approaches to partitioning E_{app} . Equation (1) is simpler in structure, but E_L appears to be a better metric quantifying interspecific relationships. However, it is not so straightforward to partition E_{app} into E_{intra} and E_L because of the covariance term

Table 2: Contributions of intra- and interspecific activation energies (eq. [1]) to the difference in apparent activation energy (E_{app}) between autotrophic and heterotrophic protists

Term	Definition	Unit	Autotrophs	Heterotrophs
n	Number of taxa		438	88
M	Total number of observations		2,719	711
E_{app} (OLSR; mean \pm SE)	Apparent activation energy calculated via OLSR (eq. [3])	eV	.378 \pm .021	.655 \pm .066
E_{inter} (mean \pm SE)	OLSR slope of $\ln(\text{normalized growth rate})$ against mean Boltzmann temperature (\bar{x})	eV	-.379 \pm .049	-.482 \pm .095
E_L (mean \pm SE)	OLSR slope of $\ln(\text{maximal growth rate})$ against optimal Boltzmann temperature (x_m)	eV	.241 \pm .036	.460 \pm .134
$\text{Var}(x)$	Variance of all Boltzmann temperatures	eV ⁻²	1.23	1.12
$\text{Var}(\bar{x})$	Variance of \bar{x}	eV ⁻²	.66	.40
$\text{Var}(x_m)$	Variance of optimal temperature	eV ⁻²	.78	.46
$\langle E_{intra} \rangle$	Variance-weighted mean E_{intra}	eV	.586 \pm .026	.841 \pm .041
$\frac{E_{inter} \text{Var}(\bar{x})}{\text{Var}(x)}$	Interspecific term in equation (1)	eV	-.203 \pm .026	-.173 \pm .040
$\frac{E_L \text{Cov}(x_m, \bar{x})}{\text{Var}(x)}$	Interspecific (second) term in equation (2)	eV	.127 \pm .021	.159 \pm .051
$\frac{\text{Cov}(E_{intra}x_m, \bar{x})}{\text{Var}(x)}$	Third term in equation (2)	eV	.296 \pm .024	.262 \pm .061
Calculated E_{app}	E_{app} calculated based on equation (1)	eV	.378 \pm .021	.654 \pm .066

Note: $\langle E_{intra} \rangle = \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{x})^2 / [M \text{Var}(x)]$, in which m_j is the number of observations of the j th taxon. The total number of observations in the pooled data set is $M = \sum_{j=1}^n m_j$. $E_{intra,j}$ is the intraspecific activation energy of the j th taxon. \bar{x} is the grand mean of x . OLSR = ordinary least squares regression.

$\text{Cov}(E_{\text{intra}}x_m, \bar{x})$, which also affects E_{intra} . If the E_{intra} of all taxa were the same, these relationships would be clearer:

$$E_{\text{app}} \approx E_{\text{intra}} \left(1 - \frac{\text{Cov}(x_m, \bar{x})}{\text{Var}(x)} \right) + E_L \frac{\text{Cov}(x_m, \bar{x})}{\text{Var}(x)},$$

$$E_L \approx E_{\text{intra}} + E_{\text{inter}} \frac{\text{Var}(\bar{x})}{\text{Cov}(x_m, \bar{x})}.$$

Furthermore, if $\text{Cov}(x_m, \bar{x}) = \text{Var}(x)$, E_{app} would equal E_L and $E_L = E_{\text{intra}} + E_{\text{inter}}$. Here, we focus on partitioning E_{app} into E_{intra} and E_{inter} but also provide results of E_L .

In summary, partitioning E_{app} allows us to address differences in thermal sensitivities and adaptation. Obtaining an E_{inter} of zero would indicate that across taxa, any adaptation to local temperature conditions has not changed the positive biochemical relationship between temperature and growth, supporting the hotter-is-better hypothesis. If this were so, then the temperature dependence of all taxa could be described by a single exponential function—that is, the linear temperature response curves in figure 1 would collapse to a single line, and $E_{\text{app}} = E_{\text{intra}}$. In contrast, obtaining a negative E_{inter} would indicate that taxa from colder environments achieve enhanced growth via adaptation to cold temperature or that high temperature constrains the growth of taxa in warm environments ($E_{\text{app}} < E_{\text{intra}}$), supporting the hotter-is-not-better hypothesis (fig. 1). The value of E_{inter} could also be positive, indicating that the difference in maximal growth rates between warm- and cold-adapted species is even greater than predicted from intraspecific relationships (the hotter-is-even-better hypothesis; Frazier et al. 2006). By applying equation (1), we can then test the extent to which the difference in the apparent temperature sensitivity (E_{app}) between auto- and heterotrophs is caused by intraspecific temperature sensitivity (E_{intra}) or interspecific thermal adaptation (E_{inter}). As explained above, here we apply this analysis to protists, but this approach can have wider utility for partitioning any trend (regression slope) into different scales.

Methods

Data Sets

We compiled a data set of per capita growth rate (μ ; d^{-1}) versus temperature (T) responses for marine and freshwater autotrophic and heterotrophic protists from published laboratory experiments (cyanobacteria were excluded). Data have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.dr7sqvb1v>; Chen et al. 2022). These organisms were originally isolated from a broad range of environments ranging from polar regions to the tropics and were cultured under optimal light and nutrient conditions. Experimental data were included if they met the

following criteria: at least three data points with positive μ and at least two unique temperatures at which positive μ were measured. To calculate E_{app} , we also removed data points with nonpositive μ and those with temperatures above the optimal growth temperature (defined as the temperature corresponding to the maximal μ), which leads to a data set with 2,719 and 711 data points for autotrophic and heterotrophic protists, respectively. Mixotrophy was not considered a confounding issue, as autotrophs were grown without prey and heterotrophs were grown in the dark or in low light. These criteria provided 438 and 88 independent experiments for auto- and heterotrophic protists, respectively (table 2).

Estimation of E_{app} via Linear Regressions

Using OLSR (lm function, R ver. 4.2.0; R Core Team 2022), equation (4) was fitted to the two pooled data sets (table 1):

$$\ln \mu = \ln \mu_r + \frac{E_{\text{app}}}{k_b} \left(\frac{1}{T_0 + T_r} - \frac{1}{T_0 + T} \right), \quad (4)$$

where T_r is a reference temperature (15°C) and T is the experimental temperature (°C), T_0 equals 0°C or 273.15 K, k_b is the Boltzmann constant (8.62×10^{-5} eV K^{-1}), and μ_r is the growth rate (day^{-1}) at T_r . The term E_{app} is the apparent activation energy without considering cell size effects, as E_{app} changed negligibly if size was included (López-Urrutia et al. 2006; Chen and Liu 2011; supplement 2). For convenience, the Boltzmann temperature was defined as

$$x = \frac{1}{k_b} \left(\frac{1}{T_0 + T_r} - \frac{1}{T_0 + T} \right),$$

with $y = \ln \mu$ and $y_r = \ln \mu_r$, so that equation (4) simplifies to $y = E_{\text{app}}x + y_r$.

Estimation of E_{intra} , E_{inter} , and E_L

For each taxon, E_{intra} was estimated via an OLSR: $y_{ij} = E_{\text{intra}j}x_{ij} + b_j$, where x_{ij} and y_{ij} are the i th Boltzmann temperature and $\ln(\text{growth rate of the } j\text{th taxon})$, respectively, and b_j is the regression intercept (i.e., $\ln(\text{growth rate})$ normalized to the reference temperature [T_r] of taxon j). The rate of how b_j decreases with \bar{x}_j , E_{inter} , was calculated by OLSR as the slope of b_j against \bar{x}_j weighted by the number of data points of each taxon, m_j (table 1; fig. 1B). The rate of how maximal growth rate (y_{mj}) decreases with optimal temperature (x_{mj}), E_L , was calculated by weighted OLSR as the slope of y_{mj} against x_{mj} , also weighted by m_j (table 1; fig. 1C). Standard errors of E_{inter} , E_L , E_{app} , and $\langle E_{\text{intra}} \rangle$ were calculated by bootstrapping (Johnson 2001).

Results

Difference in Auto- and Heterotrophic

E_{app} , E_{intra} , E_{inter} , and E_L

Analysis using equation (1) indicates a difference in apparent activation energy E_{app} between auto- and heterotrophs of 0.277 eV (table 2; fig. 2). The difference persisted even when cell size was included in the analysis (supplement 2). In addition, the E_{app} obtained from OLSR was identical to that obtained using equation (1), confirming its validity.

The 0.277 eV difference of E_{app} can be largely attributed to $\langle E_{\text{intra}} \rangle$ (0.255 eV = 92%) and $E_{\text{inter}} \text{Var}(\bar{x}) / \text{Var}(x)$ (0.030 eV = 11%), with the rest contributed by covariance terms (table 2; table S1). The term $E_{\text{inter}} \text{Var}(\bar{x}) / \text{Var}(x)$ was negative for both trophic levels, indicating that E_{app} is dampened by thermal adaptation. Neither the term $E_{\text{inter}} \text{Var}(\bar{x}) / \text{Var}(x)$ nor the term E_{inter} were significantly different between auto- and heterotrophs observed, suggesting similar thermal adaptation (across taxa) capacities.

Discussion

Current meta-analyses seem to suggest that autotrophs are less thermally sensitive than heterotrophs, implying that environmental warming will lead to metabolic and trophodynamic imbalances (Allen et al. 2005; López-Urrutia et al. 2006; Rose and Caron 2007; Chen et al. 2012). Here, we raise concerns that such evaluations conflate within- and across-taxa relationships. By developing a mathematical framework (eq. [1]) that separates within- and across-taxa thermal sensitivities, we find that previously observed apparent differences do indeed arise mostly from within-taxa responses. Across taxa, it appears that auto- and heterotrophic protists are equally capable of adapting to different thermal environments (similar E_{inter}). This suggests that photosynthetic physiology, argued to reduce autotrophic thermal sensitivity (Allen et al. 2005), contributes little to how taxa thermally adapt; that is, by inference adaptation resulting in improved performance acts on levels other than photosynthesis, which could include shifts in chaperone protein structure that may lead to salutatory changes in thermal sensitivity (Somero 2020).

Our ability—using equation (1)—to decouple and quantify the magnitude and relative contribution of thermal adaptation (E_{inter}) has relevance to an ongoing debate in thermal ecology. The hotter-is-better (or thermodynamic constraint) hypothesis argues that physiological rates are strictly driven by biochemical reactions, with taxa occupying warmer-temperature niches performing better at higher thermal optima ($E_{\text{app}} = E_{\text{intra}}$; Angilletta et al. 2010). In contrast, the hotter-is-not-better (or biochemical adaptation) hypothesis predicts that taxa in cold environments evolve to compensate for their

biochemical constraints or that the growth rates of taxa in warm environments are constrained by high temperature (Angilletta et al. 2010; Smith et al. 2019; Kontopoulos et al. 2020). Here, we show that hotter is *partially* better for both autotrophic and heterotrophic protists ($-\langle E_{\text{intra}} \rangle < E_{\text{inter}} < 0$; both E_{app} and E_L are positive; Barton and Yvon-Durocher 2019; Liu et al. 2022), suggesting that thermal adaptation across taxa has partially compensated for thermodynamic constraints in both groups.

Regarding differences in E_{intra} , in contrast to recent predictions that the difference between auto- and heterotrophs is relatively small ($\Delta E_{\text{intra}} = 0.1$ eV; Wang et al. 2019), here, using a larger data set and a more sophisticated approach, we find that the difference ($\Delta E_{\text{app}} = 0.277$ eV, $\Delta \langle E_{\text{intra}} \rangle = 0.255$ eV) is similar to previous estimates ($\Delta E_{\text{app}} = 0.33$ eV; autotrophs ≈ 0.32 eV vs. heterotrophs ≈ 0.65 eV; Allen et al. 2005; López-Urrutia et al. 2006; Chen et al. 2012; Regaudie-de-Gioux and Duarte 2012). However, equation (1), a critical finding of our work, also implies that the difference in E_{app} depends not only on E_{intra} but also on the ratio of thermal diversity to the measurement temperature variance ($\text{Var}(\bar{x}) / \text{Var}(x)$), which likely depends on the specific data set.

Admittedly, our approach (eq. [1]) has not considered how other factors, such as size, phylogeny, and resource limitation, affect growth rates and temperature sensitivities (Frazier et al. 2006; López-Urrutia et al. 2006) and is therefore a first-order estimate of partitioning E_{app} into intra- and interspecific terms. While this may limit our ability to generalize our findings beyond protists, our preliminary analyses of prokaryotes and insects show that the estimates of E_{intra} are convergent, while the estimates of E_{inter} may be confounded by other factors (table S1). Future work can tease out the effects of covariates (e.g., size) by analyzing a multiple regression model. Our approach also ignores the declining part of the TPC, which, although a common practice in estimating E_{app} , may be relevant on some occasions (Chen 2022). Consequently, given the insights provided by our work, we can consider the whole TPC when evaluating community responses to warming in the future.

In conclusion, we caution that the current perception of the lower temperature sensitivity of autotrophs than heterotrophs is based on an oversimplified analysis that indiscriminately combines intra- and interspecific trends. Our analysis does support that there is a greater intraspecific temperature sensitivity for heterotrophs than for autotrophs. However, it is necessary to consider both the within-taxa thermodynamic effect and across-taxa trends when evaluating thermal responses and quantifying differences between trophic groups. Our approach can be applied to other groups and analyses, such as phylogenetic/environmental comparisons (e.g., between terrestrial and aquatic

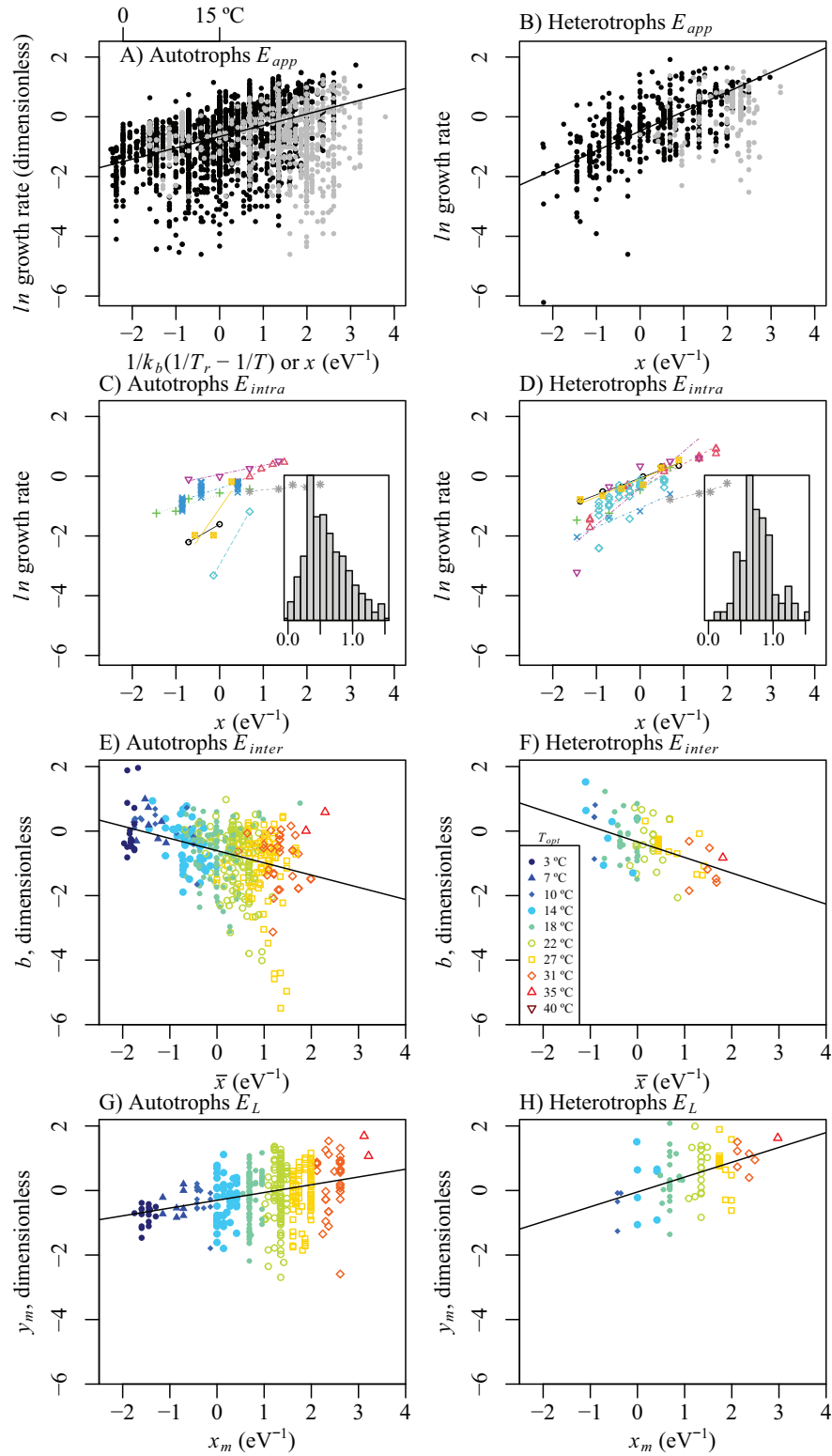


Figure 2: A, B, E_{app} estimated by ordinary least squares (OLS) linear regressions based on pooled data sets of autotrophic (A) and heterotrophic (B) protists. The gray points represent supraoptimal temperatures that are not included in the regression. C, D, Examples of OLS regressions to estimate E_{intra} of each taxon. For visual clarity, only eight taxa, randomly selected from each group, are shown. The insets show the frequency distribution of E_{intra} . E, F, E_{inter} estimated by OLS regressions of b against \bar{x} weighted by the number of data points of each taxon. The colors of data points indicate the optimal temperature (T_{opt}). G, H, E_L estimated by OLS regressions of y_m against x_m weighted by the number of data points of each taxon.

taxa or between polar and tropical taxa). It will be interesting to know whether these differences arise from intraspecific temperature sensitivities.

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Statement of Authorship

B.C.: conceptualization, funding acquisition, method development, data collection and analysis, code simulation, writing—original draft and further revision; D.J.S.M.: substantial supervision, writing—original draft and further revision; Q.W.: data collection and editing; H.L.: funding acquisition, supervision; S.M.-D.: conceptualization, funding acquisition, supervision, writing—review and editing.

Data and Code Availability

Data are available in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.dr7sqvb1v>; Chen et al. 2022). Code is available on Zenodo (<https://doi.org/10.5281/zenodo.6791293>). Both are also available on Github (<https://github.com/BingzhangChen/ActivationEnergy.git>).

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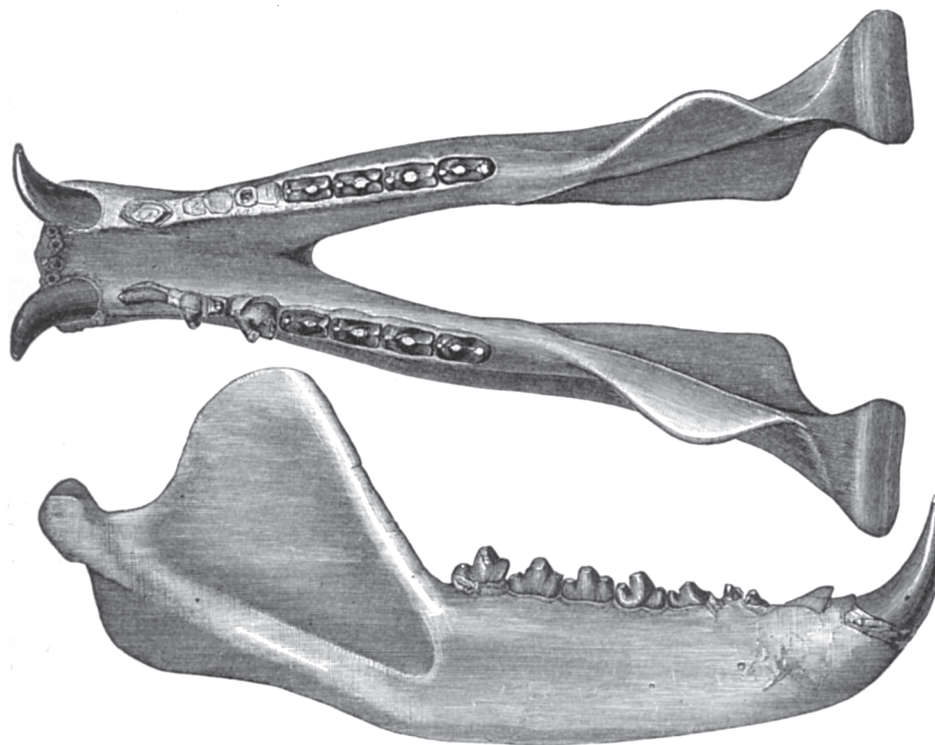
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“The appearance is that of a perissodactyle mammal, and the astragalus of the Mesonyx might well be taken for that of an ungulate of that suborder.” Figured: “Mandible of *Mesonyx ossifragus* Cope, from the Wasatch epoch of the Big Horn river, Wyoming.” From “The Creodonta” by E. D. Cope (*The American Naturalist*, 1884, 18:255–267).