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Partitioning the apparent temperature sensitivity into within- and across-taxa responses: revisiting the difference between autotrophic and heterotrophic protists

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Abstract Conventional analyses suggest 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.

Chinese title: 分离种内和种间的温度敏感性：重新审视自养和异养单细胞真核生物的差异

Abstract in Chinese:

传统研究认为异养生物代谢速率相比自养生物而言对温度变化更加敏感，因此升温会增强自养和异养过程的不平衡。然而，之前研究未曾合理区分种内和种间对温度响应的差异。本研究提出一种新的方法分离种内和种间对温度的不同响应，发现自养和异养单细胞真核生物的表观温度敏感性（活化能）的差异主要（92%）来自于种内关系。不同种对温度的适应提高了它们的生长率；然而此生长率的提高只能部分抵消种内温度对生长率的影响。本研究揭示了在比较不同生物之间温度敏感性时区分种内和种间关系的重要性。

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Introduction

If temperature rises by 30 ºC, heterotrophic respiration rates are argued to increase 16-fold while photosynthetic rates only increase 4-fold (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 (MTE) 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 to photosynthesis may increase atmospheric CO_2 and reduce ecosystems' ability 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. 2022; *see* Smith et al. 2019 for a graphical illustration of all three hypotheses).

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.

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2012). It is still unclear whether it is the difference of 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 $\mu = {dN}/{Ndt}$ in which N is the 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 bio-geochemical models related to climate change (Crichton et al. 2020).

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 to assess 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 rate measurements at any temperature. Consequently, large data sets

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can be compiled from the literature, since any measured rates at a known temperature may be included. E_{app} is then assessed by ordinary least squared regressions (OLSR, table 1; fig. 1A red line; Allen et al. 2005; López-Urrutia et al. 2006). However, this analysis conflates two processes: within-taxa (intraspecific) physiological responses and across-taxa (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 due to the greater effort required in obtaining TPCs than assembling single temperature response data. A number of previous studies also estimated E_{intra} of 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, to our knowledge, 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 cold, exhibiting rates equal (or close) to 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., $E_{app} = E_{intra} - E_{inter}$) due to the inherent variance and stochasticity as described below.

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

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

in which $\langle E_{intra} \rangle$ is the variance-weighted mean E_{intra} (table 2). x is the transformed environmental temperature (*see* Methods). $Var(x)$ is the variance of all x in the dataset. $Var(\bar{x})$ is the variance of mean x of each taxon. E_{inter} is derived from the OLSR slope of *ln* normalization rate (the intercept of the linear regression equation used to estimate E_{intra} of each taxon) against $\bar{x}$. Note that both $Var(\bar{x})$ and E_{inter} need to be weighted by the number of measurements of each taxon (Supplement 1). $Var(\bar{x})$ 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 ($Var(x_m)$; table 2). $Var(x)$ is the variance of the measurement temperatures. Therefore, the contribution of E_{inter} to E_{app} is weighted by $Var(\bar{x})/Var(x)$, 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 to the environmental temperature variability; $Var(\bar{x})/Var(x)$) would make E_{app} approach $\langle E_{intra} \rangle$. Biologically, this means that if the taxa come from similar temperature regimes with similar adaptations to those temperature regimes, then all the variation in apparent thermal sensitivity should be due entirely 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 linear regression of *ln* 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):

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

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

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in which *Cov* indicates the covariance operator.

Eq. 1 and 2 offer different approaches to partitioning E_{app}. Eq. 1 is simpler in structure, but E_L appears a better metric quantifying interspecific relationships. However, it is not so straightforward to partition E_{app} into E_{intra} and E_L due to the covariance term $Cov(E_{intra}x_m, \bar{x})$ which also affects E_{intra}. If the E_{intra} of all taxa were the same, these relationships would be clearer:

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

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

Furthermore, if $Cov(x_m, \bar{x}) = Var(x)$, E_{app} would equal E_L and $E_L = E_{intra} + E_{inter}$. 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 0 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: i.e., the linear temperature response curves in fig. 1 would collapse to a single line, and $E_{app} = E_{intra}$. 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 ($E_{app} < \langle E_{intra} \rangle$), 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 ("hotter is even better"; Frazier et al. 2006). By applying Eq. 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 as to partitioning any trend (regression slope) into different scales.

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Methods

Datasets. We compiled a dataset of *per capita* growth rate (μ, d^{-1}) vs. temperature (T) responses for marine and freshwater autotrophic and heterotrophic protists from published laboratory experiments (cyanobacteria were excluded). Data are 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 3 data points with positive μ and at least 2 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 dataset with 2719 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 4.2.0, R Core Team 2022), Eq. (4) was fit to the two pooled datasets (table 1):

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

where T_r is a reference temperature (15 ºC), and T is the experimental temperature (ºC); T_0 equals 0 ºC or 273.15 K; k_b is the Boltzmann constant (8.62×10^{-5} eV K^{-1}); μ_r is growth rate (d^{-1}) at T_r. E_{app} is the apparent activation energy, without considering cell size effects as E_{app} changed negligibly if size was included (Lopez-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 Eq. (4) simplified to $y = E_{app}x + y_r$.

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Estimation of E_{intra}, E_{inter}, and E_L. E_{intra} of each taxon was estimated via an OLSR: $y_{ij} = E_{intra,j}x_{ij} + b_j$ where x_{ij} and y_{ij} are the ith Boltzmann temperature and *ln* growth rate of the jth taxon, respectively, and b_j is the regression intercept (i.e., the *ln* growth rate normalized to the reference temperature (T_r) of taxon *j*). E_{inter}, the rate of how b_j decreases with $\bar{x}_j$, 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). E_L, the rate of how maximal growth rate (y_{mj}) decreases with optimal temperature (x_{mj}), was calculated by the 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 $\langle E_{intra} \rangle$ were calculated by bootstrapping (Johnson 2001).

Results

Difference in auto- and heterotrophic E_{app}, E_{intra}, E_{inter}, and E_L. Analysis using Eq. (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 Eq. (1), confirming its validity.

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

Discussion

Current meta-analyses reveal that autotrophs are less thermally sensitive than heterotrophs, inferring that environmental warming will lead to metabolic and trophodynamic imbalances (Allen et al. 2005; López-

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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 to adapt 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; i.e., 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 Eq. (1) – to decouple and quantify the magnitude and relative contribution of thermal adaption (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-temperatures niches performing better at higher thermal optima ($E_{app} = E_{intra}$). 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 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 auto- and heterotrophic protists ($-\langle E_{intra}\rangle < E_{inter} < 0$; 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 ($\Delta E_{intra} = 0.1$ eV; Wang et al. 2019), here using a larger data set and a more sophisticated approach, we find that the difference ($\Delta E_{app} = 0.277$ eV, $\Delta\langle E_{intra}\rangle = 0.255$ eV) is similar to previous estimates ($\Delta E_{app} = 0.33$ eV; autotrophs ≈ 0.32 eV vs. heterotrophs ≈ 0.65 eV; Allen et al. 2005; Lopez-Urrutia et al. 2006; Chen et al. 2012; Regaudie- de- Gioux and Duarte 2012). However, Eq. (1), a critical finding of our work, also implies that the difference in E_{app} not only depends on E_{intra} but

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also on the ratio of thermal diversity to the measurement temperature variance ($Var(\bar{x})/Var(x)$), which likely depends on the specific dataset.

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 on 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 taxa or between polar and tropical taxa). It will be interesting to know whether these differences arise from intraspecific temperature sensitivities or not.

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Author contributions

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 & editing.

Data and Code Accessibility

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 of them are also available on Github (https://github.com/BingzhangChen/ActivationEnergy.git).

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Tables

Table 1. A summary of regression models used in this study.

Description	Model equation	Symbol definition
OLSR on the pooled dataset of *ln* growth rate against temperature to estimate E_{app}.	$y_i = E_{app}x_i + y_r + \varepsilon_i$	y_i: *ln* growth rate of measurement *I* in the pooled dataset 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 dataset of *ln* growth rate against temperature and *ln* cell volume to estimate E_{app}.	$y_i = E_{app}x_i + \alpha_v \ln V_i + y_r + \varepsilon_i$	V_i: cell volume (μm^3) of measurement *i* in the pooled dataset. 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}.	$y_{ij} = E_{intra,j}x_{ij} + b_j + \varepsilon_{ij}$	x_{ij}: Boltzmann temperature *i* of taxon *j*. y_{ij}: *ln* growth rate of taxon *j* at temperature *i*. $E_{intra,j}$: intraspecific activation energy (eV) of taxon *j*. b_j: normalized *ln* growth rate (regression slope) of taxon *j*. 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}.	$b_j = E_{inter}\bar{x}_j + b_0 + \beta_j$ $\frac{m_j}{M}\beta_j \sim N(0, \sigma_\beta^2)$	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: the number of observations of taxon *j*. *M*: the total number of observations of the pooled dataset. 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.	$y_{m,j} = E_L x_{mj} + B_0 + v_j$ $\frac{m_j}{M}v_j \sim N(0, \sigma_v^2)$	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 $(m_j/M)v_j$.

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Table 2. Contributions of intra- and interspecific activation energies (Eq. 1) to the difference of 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		2719	711
E_{app} (OLSR; Mean±SE)	Apparent activation energy calculated via OLSR (Eq. 3)	eV	0.378±0.021	0.655±0.066
E_{inter} (Mean±SE)	OLSR slope of *ln* normalized growth rate against mean Boltzmann temperature ($\bar{x}$)	eV	-0.379±0.049	-0.482±0.095
E_L (Mean±SE)	OLSR slope of *ln* maximal growth rate against optimal Boltzmann temperature (x_m)	eV	0.241±0.036	0.460±0.134
$Var(x)$	Variance of all Boltzmann temperatures	eV^{-2}	1.23	1.12
$Var(\bar{x})$	Variance of $\bar{x}$	eV^{-2}	0.66	0.40
$Var(x_m)$	Variance of optimal temperature	eV^{-2}	0.78	0.46
$\langle E_{intra} \rangle$	Variance-weighted mean E_{intra}	eV	0.586±0.026	0.841±0.041
$E_{inter}\frac{Var(\bar{x})}{Var(x)}$	Interspecific term in Eq. (1)	eV	-0.203±0.026	-0.173±0.040
$E_L\frac{Cov(x_m,\bar{x})}{Var(x)}$	Interspecific (2nd) term in Eq. (2)	eV	0.127±0.021	0.159±0.051
$\frac{Cov(E_{intra}x_m,\bar{x})}{Var(x)}$	3rd term in Eq. (2)	eV	0.296±0.024	0.262±0.061
Calculated E_{app}	E_{app} calculated based on Eq. (1)	eV	0.378±0.021	0.654±0.066

Note: OLSR: Ordinary Least Squares. $\langle E_{intra} \rangle = \sum_{j=1}^{n} E_{intra,j} \sum_{i=1}^{m_j} \left(x_{ij} - \bar{\bar{x}}\right)^2 / [MVar(x)]$, in which m_j is the number of observations of the jth taxon. The total number of observations in the pooled dataset is $M = \sum_{j=1}^{n} m_j$. $E_{intra,j}$ is the intraspecific activation energy of the jth taxon. $\bar{\bar{x}}$: grand mean of x.

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Figure legends

Figure 1 Relationship between apparent (E_{app}, eV), intraspecific (E_{intra}), interspecific (E_{inter}), and long-term activation energy (E_L) in an idealized case of perfect thermal adaptation. In (A), black lines represent the relationship of *ln* growth rate (y) versus Boltzmann temperature (x) of 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).

Figure 2 (A, B) E_{app} estimated by OLS linear regressions based on pooled datasets of (A) autotrophic and (B) heterotrophic 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.

Figure 1

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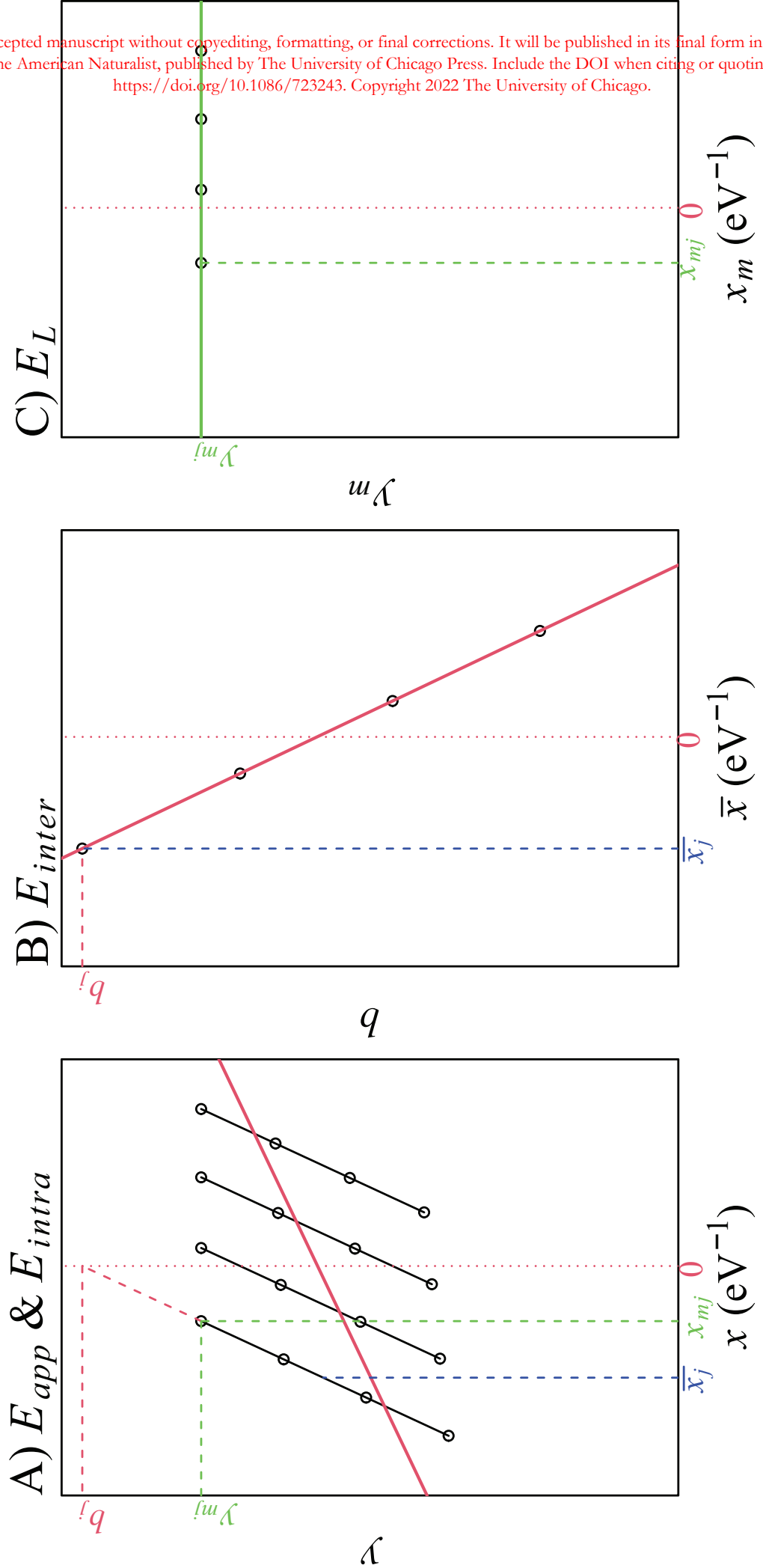

Figure 2

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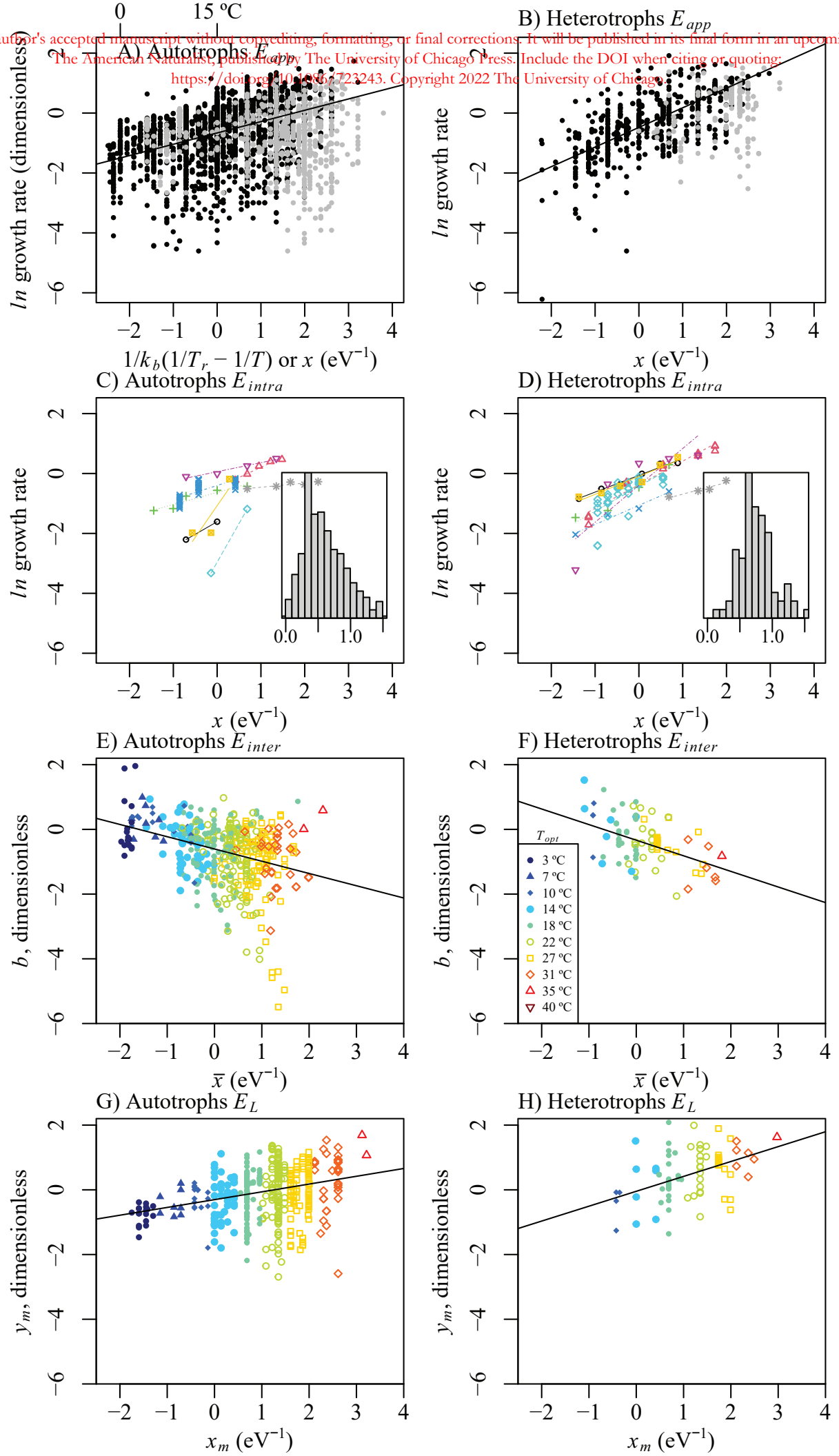

Supplementary Files (PDF, Word, TeX, figures)

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Online Supplement:

Partitioning the apparent temperature sensitivity into within- and across-taxa responses: revisiting the difference between autotrophic and heterotrophic protists

The American Naturalist

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Supplemental 1. Derivation of Eq. 1-3 in the main text and estimates of prokaryotes and insects

Derivation of Eq. 1 in the main text

We assume that there are n species for which per capita growth rates (μ, d^{-1}) are measured at different temperatures (T in Kelvin) in the pooled dataset. For species j, there are m_j paired measurements of temperature and growth rate. Because we only use the data with the temperatures below optimal growth temperature (T_{opt}) to compute E_{app}, we can assume a linear equation for the relationship between ln growth rate ($y = ln\mu$) and Boltzmann temperature ($x = \frac{1}{k_b}(\frac{1}{T_r} - \frac{1}{T})$) of each species:

$$y_{ij} = E_{intra,j}x_{ij} + b_j + \epsilon_{ij} \tag{S1}$$

in which $E_{intra,j}$ is the intraspecific activation energy of species j. y_{ij} and x_{ij} is the ith measurement of ln growth rate and Boltzmann temperature of species j. b_j is the growth rate normalized to the reference temperature T_r. ϵ_{ij} is the residual of the ith measurement of species j and has the mean of 0 and variance of σ_j^2 ($\epsilon_{ij} \sim N(0, \sigma_j^2)$).

The total number of paired observations in the pooled dataset is $M = \sum_{j=1}^{n} m_j$. The mean Boltzmann temperature of species j is $\overline{x_j} = \frac{\sum_{i=1}^{m_j} x_{ij}}{m_j}$. The grand mean Boltzmann temperature of the pooled dataset is defined as $\overline{\overline{X}} = \frac{1}{M}\sum_{j=1}^{n} m_j\overline{x_j}$.

We assume that b_j is a linear function of $\overline{x_j}$, which can be fitted via a weighed ordinary least-squares (OLS) regression shown below:

$$b_j = E_{inter}\overline{x_j} + b_0 + \beta_j \tag{S2}$$

in which E_{inter} and b_0 are the slope and intercept, respectively, that minimize the weighed sum of residual squares ($\sum_{j=1}^{n} \frac{m_j}{M}\beta_j^2$). E_{inter} can be considered as a form of interspecific activation energy as explained in the main text. We assume that the residual β_j follows a normal distribution

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with a weighed mean 0 and variance σ_β^2 (i.e., $\frac{m_j}{M}\beta_j \sim N(0, \sigma_\beta^2)$).

The mean of b_j ($\overline{b}$) can be calculated as:

$$\begin{aligned}
\overline{b} &= \frac{1}{M}\sum_{j=1}^{n} m_j b_j \\
&= \frac{1}{M}\sum_{j=1}^{n} m_j (E_{inter}\overline{x_j} + b_0 + \beta_j) \\
&= E_{inter}(\sum_{j=1}^{n} \frac{m_j}{M}\overline{x_j}) + b_0 + \sum_{j=1}^{n} \frac{m_j}{M}\beta_j \\
&= E_{inter}\overline{\overline{X}} + b_0
\end{aligned} \tag{S3}$$

The grand mean of y is $\overline{\overline{Y}} = \frac{1}{M}\sum_{j=1}^{n} m_j \overline{y_j} = \frac{1}{M}\sum_{j=1}^{n} m_j (E_{intra,j}\overline{x_j} + b_j) = \frac{1}{M}\sum_{j=1}^{n} m_j E_{intra,j}\overline{x_j} + \overline{b}$.

E_{app} is calculated as the slope of the (OLS) regression of y against x:

$$E_{app} = \frac{\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})y_{ij}}{\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})^2} \tag{S4}$$

The numerator of Eq.(S4) can be manipulated as:

$$\begin{aligned}
\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})y_{ij} &= \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})\left(E_{intra,j}x_{ij} + b_j + \epsilon_{ij}\right) \\
&= \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})\left(E_{intra,j}x_{ij} - E_{intra,j}\overline{\overline{X}} + E_{intra,j}\overline{\overline{X}} + b_j + \epsilon_{ij}\right) \\
&= \sum_{j=1}^{n} E_{intra,j}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})^2 + \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})(E_{intra,j}\overline{\overline{X}} + b_j - \overline{b}) + \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij} - \overline{\overline{X}})\epsilon_{ij}
\end{aligned} \tag{S5}$$

Because $b_j - \overline{b} = E_{inter}(\overline{x_j} - \overline{\overline{X}}) + \beta_j$, we have:

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$$\begin{aligned}\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})(E_{intra,j}\overline{\overline{X}}+b_j-\overline{b}) &= \sum_{j=1}^{n}(E_{intra,j}\overline{\overline{X}}+b_j-\overline{b}+\beta_j)\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})\\ &= \sum_{j=1}^{n}(E_{intra,j}\overline{\overline{X}}+b_j-\overline{b}+\beta_j)m_j(\overline{x_j}-\overline{\overline{X}})\\ &= \sum_{j=1}^{n}\left[E_{intra,j}\overline{\overline{X}}+E_{inter}(\overline{x_j}-\overline{\overline{X}})+\beta_j\right]m_j(\overline{x_j}-\overline{\overline{X}})\\ &= \overline{\overline{X}}\sum_{j=1}^{n}m_jE_{intra,j}(\overline{x_j}-\overline{\overline{X}})+E_{inter}\sum_{j=1}^{n}m_j(\overline{x_j}-\overline{\overline{X}})^2+\sum_{j=1}^{n}m_j\beta_j(\overline{x_j}-\overline{\overline{X}})\end{aligned} \tag{S6}$$

We can show that:

$$\begin{aligned}\sum_{j=1}^{n}\frac{m_j}{M}E_{intra,j}(\overline{x_j}-\overline{\overline{X}}) &= \sum_{j=1}^{n}\frac{m_j}{M}(E_{intra,j}-\overline{E_{intra}})(\overline{x_j}-\overline{\overline{X}})\\ &= Cov(E_{intra},\overline{x})\end{aligned} \tag{S7}$$

in which $\overline{E_{intra}}=\frac{\sum_{j=1}^{n}m_jE_{intra,j}}{M}$. $Cov(E_{intra},\overline{x})$ is the covariance between $E_{intra,j}$ and $\overline{x_j}$ with unequal probability $\frac{m_j}{M}$. Similarly, $\sum_{j=1}^{n}\frac{m_j}{M}(\overline{x_j}-\overline{\overline{X}})^2$ is the variance of $\overline{x_j}$ and $\sum_{j=1}^{n}\frac{m_j}{M}\beta_j(\overline{x_j}-\overline{\overline{X}})$ is the covariance between β_j and $\overline{x_j}$ ($Cov(\beta,\overline{x})$), both with unequal probabilities $\frac{m_j}{M}$.

Similarly, the last term at the right-hand side of Eq. S5 becomes:

$$\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})\epsilon_{ij}=MCov(\epsilon,x) \tag{S8}$$

Therefore, E_{app} can be decomposed as:

$$E_{app}=\frac{\sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2}{MVar(x)}+E_{inter}\frac{Var(\overline{x})}{Var(x)}+\overline{\overline{X}}\frac{Cov(E_{intra},\overline{x})}{Var(x)}+\frac{Cov(\beta,\overline{x})}{Var(x)}+\frac{Cov(\epsilon,x)}{Var(x)} \tag{S9}$$

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Eq. S9 can be interpreted as follows. $\langle E_{intra} \rangle = \frac{\sum_{j=1}^{n} E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \overline{\overline{X}})^2}{MVar(x)}$ is the variance-weighted mean intraspecific activation energy. $E_{inter} \frac{Var(\overline{x})}{Var(x)}$ represents the contribution of interspecific thermal adaptation to E_{app}. The covariance term of $Cov(E_{intra}, \overline{x})$ can be interpreted as the contribution of the covariance of E_{intra} and $\overline{x}$ to E_{app}. If warm adapted species tends to have a greater E_{intra}, E_{app} will be greater, and vice versa. The second covariance term ($Cov(\beta, \overline{x})$) can be interpreted as the contribution of covariance between β, the residuals of the linear regression of log growth rate normalized to a reference temperature (b_j) against mean temperature ($\overline{x_j}$)) of each taxon, and $\overline{x}$. In other words, if the relationship between b_j and $\overline{x_j}$ is curvilinearly convex, E_{app} will be greater, and vice versa. Likewise, $Cov(\epsilon, x)$ is the covariance between ϵ, the residuals of each individual growth rate in each OLSR that estimated E_{intra} for each taxon, and temperature (x). If in general the relationship between log growth rate and temperature within each taxon is curvilinearly convex, the final E_{app} will be greater, and vice versa.

Note that because $Cov(E_{intra}, \overline{x})$, $Cov(\beta, \overline{x})$ and $Cov(\epsilon, x)$ are negligible compared to the first two terms at the right side in our datasets, Eq. S9 can be approximated as:

$$E_{app} \approx \langle E_{intra} \rangle + E_{inter} \frac{Var(\overline{x})}{Var(x)} \tag{S10}$$

Relationship between E_{app}, E_L, and E_{inter} (Eq. 2 & 3 in the main text)

The interspecific (long-term) activation energy can also be expressed by the slope of ln maximal growth rate ($y_m = ln\mu_m$) and Boltzmann optimal temperature (x_m):

$$y_{m,j} = E_L x_{m,j} + B_0 + \nu_j \tag{S11}$$

in which $y_{m,j}$ is the maximal ln growth rate of species j. $x_{m,j}$ is the Boltzmann optimal temperature of species j. E_L is the regression slope of the OLS regression line between y_m and x_m weighed by the number of measurements m_j. E_L is often used as the interspecific (long-term) activation energy in the literature (Smith et al. 2019). E_L differs from E_{inter} in that E_L is zero in the case of

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perfect adaptation ($E_{inter} < 0$) and equals to E_{intra} if there is no adaptation at all ($E_{inter} = 0$). B_0 is the regression intercept which is a constant. ν_j is the residual of species j which follows a normal distribution with a weighed mean of zero and variance of σ_ν^2 (i.e., $\frac{m_j}{M}\nu_j \sim N(0, \sigma_\nu^2)$).

To examine the relationship between E_{app} and E_L, we express the ln growth rate at the ith temperature of species j as a function of $x_{m,j}$ and $y_{m,j}$ instead of Eq. S1:

$$y_{i,j} = E_{intra,j}(x_{ij} - x_{m,j}) + y_{m,j} + \xi_{ij} \tag{S12}$$

in which x_{ij}, y_{ij}, $E_{intra,j}$, $x_{m,j}$ and $y_{m,j}$ are the same as defined in Eq. S1 and S11. ξ_{ij} is the residual of the ith measurement of species j and follows a normal distribution ($\xi_{ij} \sim N(0, \sigma_{\xi,j}^2)$).

To prepare for the following derivation, we need to define the average of $x_{m,j}$ ($\overline{X_m}$) and the average of $y_{m,j}$ ($\overline{Y_m}$) as $\overline{X_m} = \frac{1}{M}\sum_{j=1}^{n} m_j x_{m,j}$ and $\overline{Y_m} = \frac{1}{M}\sum_{j=1}^{n} m_j y_{m,j}$, respectively.

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The numerator of Eq. S4 can be rewritten as:

$$
\begin{aligned}
\sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})y_{ij} &= \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})\Big[E_{intra,j}(x_{ij}-x_{m,j})+y_{m,j}+\xi_{ij}\Big] \\
&= \sum_{j=1}^{n}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})\Big[E_{intra,j}(x_{ij}-\overline{\overline{X}})+E_{intra,j}(\overline{\overline{X}}-x_{m,j})+y_{m,j}+\xi_{ij}\Big] \\
&= \sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2-\sum_{j=1}^{n}E_{intra,j}(x_{m,j}-\overline{\overline{X}})\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}}) \\
&+\sum_{j=1}^{n}y_{m,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})+\sum_{j=1}^{n}\sum_{i=1}^{m_j}\xi_{ij}(x_{ij}-\overline{\overline{X}}) \\
&= \sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2-\sum_{j=1}^{n}m_jE_{intra,j}(x_{m,j}-\overline{\overline{X}})(\overline{x_j}-\overline{\overline{X}}) \\
&+\sum_{j=1}^{n}m_jy_{m,j}(\overline{x_j}-\overline{\overline{X}})+\sum_{j=1}^{n}\sum_{i=1}^{m_j}\xi_{ij}(x_{ij}-\overline{\overline{X}}) \\
&= \sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2-\sum_{j=1}^{n}m_jE_{intra,j}(x_{m,j}-\overline{\overline{X}})(\overline{x_j}-\overline{\overline{X}}) \\
&+\sum_{j=1}^{n}m_j(E_Lx_{m,j}+B_0+\nu_j)(\overline{x_j}-\overline{\overline{X}})+\sum_{j=1}^{n}\sum_{i=1}^{m_j}\xi_{ij}(x_{ij}-\overline{\overline{X}}) \\
&= \sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2-\sum_{j=1}^{n}m_jE_{intra,j}x_{m,j}(\overline{x_j}-\overline{\overline{X}})+\overline{\overline{X}}\sum_{j=1}^{n}m_jE_{intra,j}(\overline{x_j}-\overline{\overline{X}}) \\
&+E_L\sum_{j=1}^{n}m_jx_{m,j}(\overline{x_j}-\overline{\overline{X}})+\sum_{j=1}^{n}m_j\nu_j(\overline{x_j}-\overline{\overline{X}})+\sum_{j=1}^{n}\sum_{i=1}^{m_j}\xi_{ij}(x_{ij}-\overline{\overline{X}}) \\
&= \sum_{j=1}^{n}E_{intra,j}\sum_{i=1}^{m_j}(x_{ij}-\overline{\overline{X}})^2 \\
&+M\Big[E_LCov(x_m,\overline{x})-Cov(E_{intra}x_m,\overline{x})+\overline{\overline{X}}Cov(E_{intra},\overline{x}))+Cov(\nu,\overline{x})+Cov(\xi,\overline{x})\Big]
\end{aligned}
\tag{S13}
$$

Thus, E_{app} can be expressed as:

$$
E_{app}=\langle E_{intra}\rangle+E_L\frac{Cov(x_m,\overline{x})}{Var(x)}-\frac{Cov(E_{intra}x_m,\overline{x})}{Var(x)}+\overline{\overline{X}}\frac{Cov(E_{intra},\overline{x})}{Var(x)}+\frac{Cov(\nu,\overline{x})}{Var(x)}+\frac{Cov(\xi,x)}{Var(x)}
\tag{S14}
$$

Realizing that the three terms, $Cov(E_{intra},\overline{x})$, $Cov(\nu,\overline{x})$, and $Cov(\xi,x)$ are negligible compared

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to $Var(x)$ (Table S1), Eq. S14 can be approximated as:

$$E_{app} \approx \langle E_{intra} \rangle + E_L \frac{Cov(x_m, \overline{x})}{Var(x)} - \frac{Cov(E_{intra} x_m, \overline{x})}{Var(x)} \tag{S15}$$

By comparing Eq. S15 with Eq. S10, we can obtain an approximate relationship between E_L and E_{inter}:

$$E_L \approx \frac{E_{inter} Var(\overline{x}) + Cov(E_{intra} x_m, \overline{x})}{Cov(x_m, \overline{x})} \tag{S16}$$

Estimates of covariance terms in Eq. S9 and Eq. S14 in autotrophic and heterotrophic prokaryotes and protists as well as insects

As described in the main text, we also applied the above framework onto autotrophic and heterotrophic prokaryotes as well as insects besides the dataset of protists. The dataset of autotrophic prokaryotes (i.e., cyanobacteria) was compiled at the same time as that of the autotrophic protists (Chen and Laws 2017; Kremer et al. 2017). The dataset of heterotrophic prokaryotes was obtained from Smith et al. (2019). The dataset of insects was obtained from Rezende and Bozinovic (2019).

Table S1 shows that the covariance terms in Eq. S9 and Eq. S14 are usually negligible for all five groups of taxa, although the terms of $\frac{Cov(v,\overline{x})}{Var(x)}$ tend to be greater than other terms which is another reason that Eq. S9 is preferred over Eq. S14.

Estimates of E_{app}, E_{intra}, E_{inter} and E_L and other relevant terms in autotrophic and heterotrophic prokaryotes and insects

The following Table S2 shows the estimated terms in Eq. S10 and the simplified Eq. S14 of autotrophic and heterotrophic prokaryotes and insects. The results show that E_{app} appears similar between autotrophic and heterotrophic prokaryotes, which results from an even greater Intraspe-

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Table S1. Estimates of covariance terms in Eq. S9 and Eq. S14 for autotrophic and heterotrophic prokaryotes and protists as well as insects.

Term	Autotrophic protists	Heterotrophic protists	Autotrophic prokaryotes	Heterotrophic prokaryotes	Insects
$\overline{\overline{X}}\frac{Cov(E_{intra},\overline{x})}{Var(x)}$	-0.005	-0.01	0.01	0.002	-0.025
$\frac{Cov(\beta,\overline{x})}{Var(x)}$	6.0×10^{-18}	1.2×10^{-17}	-2.5×10^{-17}	1.8×10^{-17}	-2.1×10^{-17}
$\frac{Cov(\nu,\overline{x})}{Var(x)}$	-0.033	-0.07	0.01	-0.086	-0.11
$\frac{Cov(\xi,x)}{Var(x)}$	3.2×10^{-18}	7.7×10^{-19}	-3.3×10^{-18}	-3.2×10^{-18}	-5.1×10^{-18}
$\frac{Cov(\epsilon,x)}{Var(x)}$	2.2×10^{-18}	-2.9×10^{-18}	-7.9×10^{-18}	-1.9×10^{-18}	-7.2×10^{-19}

cific activation energy ($\langle E_{intra} \rangle$) in autotrophic prokaryotes than in heterotrophic prokaryotes but a stronger thermal adaptation (i.e., a more negative E_{inter}) in autotrophic prokaryotes. Note that Smith et al. (2019) did not observe a thermal adaptation in mesophilic bacteria either.

While the estimates of E_{app}, E_{inter}, and E_L of insects appear to be biased by other confounding factors such as body size, the estimate of E_{intra} is consistent with previous studies (Frazier et al. 2006).

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Table S2. Estimates of various terms in Eq. S9 and Eq. S14 for autotrophic and heterotrophic prokaryotes and insects.

Term	Definition	Autotrophic prokaryotes	Heterotrophic prokaryotes	Insects
n	Number of taxa	145	81	45
M	Total number of paired observations	703	1300	185
E_{app} (OLSR; Mean $\pm$ SE)	Apparent activation energy calculated via OLSR	0.63 $\pm$ 0.03	0.69 $\pm$ 0.21	0.30 $\pm$ 0.08
E_{inter} (Mean $\pm$ SE)	OLSR slope of ln normalized growth rate against $\overline{x}$	-0.32 $\pm$ 0.15	0.04 $\pm$ 0.44	-1.38 $\pm$ 0.22
E_L (Mean $\pm$ SE)	OLSR slope of y_m against x_m	0.53 $\pm$ 0.09	1.02 $\pm$ 0.36	-0.65 $\pm$ 0.17
$\langle E_{intra} \rangle$	Variance weighted mean E_{intra}	0.76 $\pm$ 0.04	0.67 $\pm$ 0.06	0.89 $\pm$ 0.03
$E_{inter}\frac{Var(\overline{x})}{Var(x)}$	Interspecific term in Eq. S9	-0.14 $\pm$ 0.07	0.02 $\pm$ 0.20	-0.57 $\pm$ 0.08
$E_L\frac{Cov(x_m,\overline{x})}{Var(x)}$	Interspecific (2nd) term in Eq. S14	0.22 $\pm$ 0.04	0.33 $\pm$ 0.14	-0.22 $\pm$ 0.06
$Var(x)$	Variance of x	0.88	1.73	0.58
$Var(\overline{x})$	Variance of $\overline{x}$	0.40	0.81	0.24
$Var(x_m)$	Variance of x_m	0.47	0.93	0.19
$\frac{Cov(E_{intra}x_m,\overline{x})}{Var(x)}$	3rd term in Eq. S14	0.37 $\pm$ 0.07	0.43 $\pm$ 0.07	0.24 $\pm$ 0.06
Calculated E_{app}	E_{app} calculated based on Eq. S9	0.62 $\pm$ 0.03	0.69 $\pm$ 0.06	0.32 $\pm$ 0.03

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Supplement 2. Estimation of E_{app} by considering the effect of cell size

To consider the effect of cell size, we constructed a multiple linear regression model following the notations in the main text:

$$y = E_{app}x + \alpha V + y_r$$

in which V is the cell volume (μm^3) and α is the size scaling coefficient of the growth rate, determining how phytoplankton growth rate changes with V after controlling the temperature effect. The detailed results are shown below in Table S3.

Table S3. Estimated E_{app} and α (Mean ± SE) of both autotrophs and heterotrophs by considering the effect of cell size. N: number of data points used in regression.

	E_{app} (eV)	α (μm^{-3})	N	R^2
Autotrophs	0.35 ± 0.01	-0.082 ± 0.006	2661	0.24
Heterotrophs	0.71 ± 0.02	-0.079 ± 0.010	704	0.54