

Online Supplement:

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

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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:

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

in which $E_{intra,j}$ is the intraspecific activation energy of species j . y_{ij} and x_{ij} is the i^{th} 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 i^{th} 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 $\bar{x}_j = \frac{\sum_{i=1}^{m_j} x_{ij}}{m_j}$. The grand mean Boltzmann temperature of the pooled dataset is defined as $\bar{\bar{X}} = \frac{1}{M} \sum_{j=1}^n m_j \bar{x}_j$.

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

$$b_j = E_{inter}\bar{x}_j + b_0 + \beta_j \quad (S2)$$

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

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 (\bar{b}) can be calculated as:

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

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

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

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

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

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

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

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

We can show that:

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

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

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

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

Therefore, E_{app} can be decomposed as:

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

Eq. S9 can be interpreted as follows. $\langle E_{intra} \rangle = \frac{\sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{x})^2}{MVar(x)}$ is the variance-weighted mean intraspecific activation energy. $E_{inter} \frac{Var(\bar{x})}{Var(x)}$ represents the contribution of interspecific thermal adaptation to E_{app} . The covariance term of $Cov(E_{intra}, \bar{x})$ can be interpreted as the contribution of the covariance of E_{intra} and \bar{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 ($Cov(\beta, \bar{x})$) 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 (\bar{x}_j) of each taxon, and \bar{x} . In other words, if the relationship between b_j and \bar{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 $Cov(E_{intra}, \bar{x})$, $Cov(\beta, \bar{x})$ 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:

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

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):

$$y_{m,j} = E_L x_{m,j} + B_0 + v_j \quad (S11)$$

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

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. v_j is the residual of species j which follows a normal distribution with a weighed mean of zero and variance of σ_v^2 (i.e., $\frac{m_j}{M}v_j \sim N(0, \sigma_v^2)$).

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

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

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 i^{th} 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.

The numerator of Eq. S4 can be rewritten as:

$$\begin{aligned}
 \sum_{j=1}^n \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}}) y_{ij} &= \sum_{j=1}^n \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}}) \left[E_{intra,j} (x_{ij} - x_{m,j}) + y_{m,j} + \xi_{ij} \right] \\
 &= \sum_{j=1}^n \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}}) \left[E_{intra,j} (x_{ij} - \bar{\bar{X}}) + E_{intra,j} (\bar{\bar{X}} - x_{m,j}) + y_{m,j} + \xi_{ij} \right] \\
 &= \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}})^2 - \sum_{j=1}^n E_{intra,j} (x_{m,j} - \bar{\bar{X}}) \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}}) \\
 &\quad + \sum_{j=1}^n y_{m,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}}) + \sum_{j=1}^n \sum_{i=1}^{m_j} \xi_{ij} (x_{ij} - \bar{\bar{X}}) \\
 &= \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}})^2 - \sum_{j=1}^n m_j E_{intra,j} (x_{m,j} - \bar{\bar{X}}) (\bar{x}_j - \bar{\bar{X}}) \\
 &\quad + \sum_{j=1}^n m_j y_{m,j} (\bar{x}_j - \bar{\bar{X}}) + \sum_{j=1}^n \sum_{i=1}^{m_j} \xi_{ij} (x_{ij} - \bar{\bar{X}}) \\
 &= \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}})^2 - \sum_{j=1}^n m_j E_{intra,j} (x_{m,j} - \bar{\bar{X}}) (\bar{x}_j - \bar{\bar{X}}) \\
 &\quad + \sum_{j=1}^n m_j (E_L x_{m,j} + B_0 + v_j) (\bar{x}_j - \bar{\bar{X}}) + \sum_{j=1}^n \sum_{i=1}^{m_j} \xi_{ij} (x_{ij} - \bar{\bar{X}}) \\
 &= \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}})^2 - \sum_{j=1}^n m_j E_{intra,j} x_{m,j} (\bar{x}_j - \bar{\bar{X}}) + \bar{\bar{X}} \sum_{j=1}^n m_j E_{intra,j} (\bar{x}_j - \bar{\bar{X}}) \\
 &\quad + E_L \sum_{j=1}^n m_j x_{m,j} (\bar{x}_j - \bar{\bar{X}}) + \sum_{j=1}^n m_j v_j (\bar{x}_j - \bar{\bar{X}}) + \sum_{j=1}^n \sum_{i=1}^{m_j} \xi_{ij} (x_{ij} - \bar{\bar{X}}) \\
 &= \sum_{j=1}^n E_{intra,j} \sum_{i=1}^{m_j} (x_{ij} - \bar{\bar{X}})^2 \\
 &\quad + M \left[E_L \text{Cov}(x_m, \bar{x}) - \text{Cov}(E_{intra} x_m, \bar{x}) + \bar{\bar{X}} \text{Cov}(E_{intra}, \bar{x}) + \text{Cov}(v, \bar{x}) + \text{Cov}(\xi, \bar{x}) \right]
 \end{aligned}
 \tag{S13}$$

Thus, E_{app} can be expressed as:

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

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

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

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

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

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

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 $\frac{Cov(v, \bar{x})}{Var(x)}$ 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-

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
$\overline{X} \frac{Cov(E_{intra}, \bar{x})}{Var(x)}$	-0.005	-0.01	0.01	0.002	-0.025
$\frac{Cov(\beta, \bar{x})}{Var(x)}$	6.0×10^{-18}	1.2×10^{-17}	-2.5×10^{-17}	1.8×10^{-17}	-2.1×10^{-17}
$\frac{Cov(v, \bar{x})}{Var(x)}$	-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).

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 ± 0.03	0.69 ± 0.21	0.30 ± 0.08
E_{inter} (Mean \pm SE)	OLSR slope of \ln normalized growth rate against \bar{x}	-0.32 ± 0.15	0.04 ± 0.44	-1.38 ± 0.22
E_L (Mean \pm SE)	OLSR slope of y_m against x_m	0.53 ± 0.09	1.02 ± 0.36	-0.65 ± 0.17
$\langle E_{intra} \rangle$	Variance weighted mean E_{intra}	0.76 ± 0.04	0.67 ± 0.06	0.89 ± 0.03
$E_{inter} \frac{Var(\bar{x})}{Var(x)}$	Interspecific term in Eq. S9	-0.14 ± 0.07	0.02 ± 0.20	-0.57 ± 0.08
$E_L \frac{Cov(x_m, \bar{x})}{Var(x)}$	Interspecific (2 nd) term in Eq. S14	0.22 ± 0.04	0.33 ± 0.14	-0.22 ± 0.06
$Var(x)$	Variance of x	0.88	1.73	0.58
$Var(\bar{x})$	Variance of \bar{x}	0.40	0.81	0.24
$Var(x_m)$	Variance of x_m	0.47	0.93	0.19
$\frac{Cov(E_{intra} x_m, \bar{x})}{Var(x)}$	3 rd term in Eq. S14	0.37 ± 0.07	0.43 ± 0.07	0.24 ± 0.06
Calculated E_{app}	E_{app} calculated based on Eq. S9	0.62 ± 0.03	0.69 ± 0.06	0.32 ± 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 \pm 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