Integrative and Comparative Biology

Integrative and Comparative Biology, volume 60, number 2, pp. 361–374
doi:10.1093/icb/icaa052

Society for Integrative and Comparative Biology

SYMPOSIUM

Thermal Responses Differ across Levels of Biological Organization

Erik N. K. Iverson,* Rachel Nix,† Ash Abebe‡ and Justin C. Havird [1,*]

*Department of Integrative Biology, The University of Texas at Austin, Austin, TX 78712, USA; †Hankamer School of Business, Baylor University, Waco, TX 76798, USA; ‡Department of Mathematics & Statistics, Auburn University, Auburn, AL 36849, USA

From the symposium "Building Bridges from Genome to Phenome: Molecules, Methods and Models" presented at the annual meeting of the Society for Integrative and Comparative Biology January 3–7, 2020 at Austin, Texas.

[1]E-mail: jhavird@utexas.edu

Synopsis Temperature is one of the most important environmental factors driving the genome-to-phenome relationship. Metabolic rates and related biological processes are predicted to increase with temperature due to the biophysical laws of chemical reactions. However, selection can also act on these processes across scales of biological organization, from individual enzymes to whole organisms. Although some studies have examined thermal responses across multiple scales, there is no general consensus on how these responses vary depending on the level of organization, or whether rates actually follow predicted theoretical patterns such as Arrhenius-like exponential responses or thermal performance curves (TPCs) that show peak responses. Here, we performed a meta-analysis on studies of ectotherms where biological rates were measured across the same set of temperatures, but at multiple levels of biological organization: enzyme activities, mitochondrial respiration, and/or whole-animal metabolic rates. Our final dataset consisted of 235 pairwise comparisons between levels of organization from 13 publications. Thermal responses differed drastically across levels of biological organization, sometimes showing completely opposite patterns. We developed a new effect size metric, "organizational disagreement" (OD) to quantify the difference in responses among levels of biological organization. Overall, rates at higher levels of biological organization (e.g., whole animal metabolic rates) increased more quickly with temperature than rates at lower levels, contrary to our predictions. Responses may differ across levels due to differing consequences of biochemical laws with increasing organization or due to selection for different responses. However, taxa and tissues examined generally did not affect OD. Theoretical TPCs, where rates increase to a peak value and then drop, were only rarely observed (12%), possibly because a broad range of test temperatures was rarely investigated. Exponential increases following Arrhenius predictions were more common (29%). This result suggests a classic assumption about thermal responses in biological rates is rarely observed in empirical datasets, although our results should be interpreted cautiously due to the lack of complete thermal profiles. We advocate for authors to explicitly address OD in their interpretations and to measure thermal responses across a wider, more incremental range of temperatures. These results further emphasize the complexity of connecting the genome to the phenome when environmental plasticity is incorporated: the impact of the environment on the phenotype can depend on the scale of organization considered.

Introduction

Temperature is one of the most important environmental factors impacting the relationship between genome and phenome. The impacts of temperature on the phenotype occur at all biological scales and can vary depending on the genotype (i.e., G × E interactions; Grishkevich and Yanai 2013). A key driver in this relationship is the temperature-dependence of metabolic rate. Metabolic rate reflects both the consumption of chemical energy by an organism and the capacity to do work. Metabolic rate is determined partly by body temperature, which varies with ambient temperature for ectotherms. When metabolic rate is elevated by ambient temperature, it can lead to faster growth, more energetic lifestyles, higher mutation rates, and even effects at the ecosystem level (Brown et al. 2004; Duarte 2007; Schulte 2015).

Advance Access publication June 1, 2020

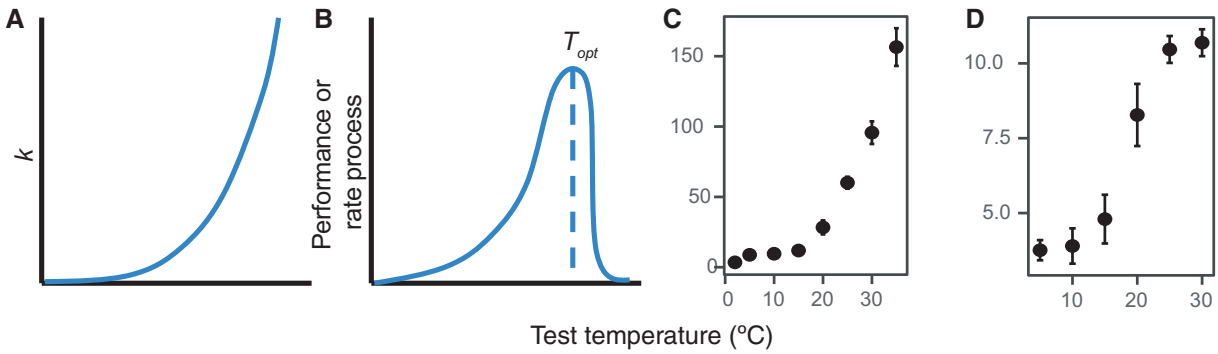

Fig. 1 (**A**) Prediction of the reaction rate (*k*) of an enzyme from the Arrhenius equation. (**B**) A theoretical TPC depicting performance or rate over temperature, a function of Arrhenius-like processes at lower temperatures followed by some form of limitation at higher ones. (**C** and **D**) Data from Fangue et al. (2009) on "background" mitochondrial respiration (C) and standard metabolic rate (D) for killifish (*F. heteroclitus*) acclimated to 5°C. Note that standard metabolic rate, an organism-level metric, could not be assayed above 30°C due to mortality, suggesting the high rates at 30°C may be the "peak" of a TPC.

One way to explain these effects is as a direct consequence of the physical laws governing the thermo-kinetics of chemical reactions along with the scaling properties of animal bodies. The expectation that biological rates should follow Arrhenius-like reaction norms (Arrhenius 1915; Fig. 1A) led to the proposal of an equation for Universal Temperature Dependence (UTD; Gillooly et al. 2001), which calculates the metabolic rate (Q) from body mass (M), absolute temperature (T), Boltzman's constant (k), a taxonomic scalar (b_0), and the activation energy of the rate-limiting step of metabolism (E):

$$Q = b_0 M^{3/4} e^{-E/kT}$$

As others have noted, UTD in its strictest form assumes that whole-organism metabolic rates are directly dependent upon biochemistry and offers no insight into ecologically or evolutionarily driven differences in metabolism contained in the taxon-specific scalar (Clarke 2004; Clarke and Fraser 2004). However, experimental evidence has demonstrated important metabolic differences among species and across levels of organization (Dell et al. 2011; Gangloff and Telemeco 2018). This suggests UTD in its strictest form is an oversimplification. For example, differences in mitochondrial efficiency (Clarke and Fraser 2004), mitochondrial density (Preedy et al. 1988), and relative tissue masses (Daan et al. 1990) may alter predictions based purely on biochemical laws. While differences might also be caused by differing enzyme activation energies under the UTD (e.g., Somero 1995), it is likely that selection at higher levels of organization has shaped thermal responses in biological rates, implying that these relationships cannot be predicted solely from biophysical laws.

To move beyond a strictly biophysical UTD, theories of evolutionary trade-offs (Clarke 1993, 2004) attempt to explain how the impact of temperature on metabolic rates is mediated by both passive biochemical laws and thermal adaptations of species living at different temperatures. For instance, the hypothesis of oxygen and capacity-limited thermal tolerance (OCLTT) gives a mechanistic explanation for the drop in organismal performance that occurs at high temperatures without relying simply on protein denaturation as an explanation, instead invoking an imbalance between oxygen supply and demand in peripheral tissues (Pörtner 2002, 2010; Schröer et al. 2011; Pörtner et al. 2017). For some organisms, processes operating at the enzymatic, mitochondrial, and whole-organism scale appear to be involved in an adaptive response of rates to temperature that differs from the expectation from pure biochemistry (White et al. 2012; Gangloff and Telemeco 2018).

These specific scales of organization should be mechanistically linked: metabolic rates are reflected in oxygen consumption, which is driven by mitochondria, which are dependent on the coordination of mitochondrial enzymes (Fig. 2). However, whether processes respond similarly to temperature across these scales of organization is unclear. For instance, Fangue et al. (2009) found that mitochondrial respiration in killifish (*Fundulus heteroclitus*) had a clearly exponential relationship with temperature, while whole-animal respiration rose but then leveled off over the same temperature range (Fig. 1).

One common framework for assessing temperature-dependence in biological rates is the use of thermal performance curves (TPCs; Fig. 1B). A TPC is constructed by measuring either a metric

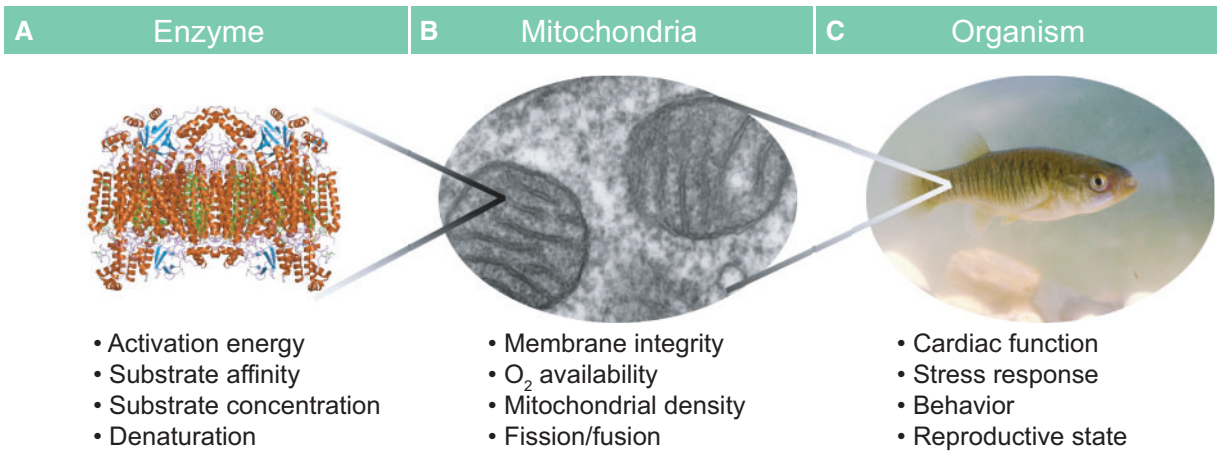

Fig. 2 Thermal response of metabolism in a killifish (*Fundulus heteroclitus*) at increasing levels of organization: (**A**) the cytochrome c oxidase enzyme (COX), which catalyzes oxidative phosphorylation, (**B**) the hepatic mitochondria in which the enzyme is embedded, and (**C**) the whole organism in its thermal environment. Listed are properties of each level that are sensitive to temperature and may play a role in shaping TPCs. Images are in the public domain or available from Wikimedia under Creative Commons license Attribution 2.5 Generic (CC BY 2.5). Image authors: (A) Jawahar Swaminathan, (B) Louisa Howard, (C) Brian Gratwicke. https://commons.wikimedia.org/wiki/File:Fundulus_heteroclitus.jpg.

of performance or a biological rate process such as oxygen consumption for an animal across a wide range of temperatures (Schulte et al. 2011; Schulte 2015; Gangloff and Telemeco 2018; Rezende and Bozinovic 2019). A typical assumption of TPCs is their asymmetrical "hump" shape: an organism displays increasing performance with temperature up to a point (T_{opt}), beyond which it falls dramatically. Hump-shaped TPCs have been fit to data at many levels of organization under the assumption that any biological rate process reflects to some degree the underlying thermo-kinetic increase in rates followed by protein denaturation or death at extreme temperatures (Schulte 2015; Gangloff and Telemeco 2018; Rezende and Bozinovic 2019). However, it is unclear how realistic this assumption is for biological rates across different levels of organization (Gangloff and Telemeco 2018). It has been noted that thermal constraints on rate become apparent at the highest level of organization (the whole organism) before lower levels (tissues, cells, and enzymes) (Pörtner 2002). The rising leg of an organism's metabolic rate may also assume a logarithmic rather than exponential shape due to temperature-dependent constraints operating at levels above that of the enzyme (Schulte et al. 2011). An organism's capacity for acclimation, a form of active plasticity, can also cause rates to show a linear relationship with temperature rather than an exponential one, if the organism compensates for the passive rise in rate with temperature (Schulte et al. 2011; Seebacher et al. 2015; Havird et al. 2020). Finally, the falling leg of the TPC may be undersampled due to the high mortality/dysfunction of animals at extreme temperatures (Penney et al. 2014; Fangue et al. 2009).

Here, we performed a meta-analysis of the temperature-dependence of biological rates across three levels of organization: activity of individual enzymes, oxygen consumption in isolated mitochondria, and whole animal metabolic rates. These represent linked components of aerobic metabolism, a process often thought to display a "typical" TPC but for which the mechanistic basis of thermal dependence is debated (Schulte 2015). Theory suggests that increasing complexity decreases optimum temperature and flattens the exponential reaction norm as mitochondrial, cellular, tissue-level, and organismal-level limits are reached. We thus predicted that rates would increase across the same set of temperatures to a lesser degree at higher levels of organization (e.g., whole organisms) compared to individual enzymes (which may show strictly Arrhenius-like exponential increases within an animal's critical thermal range). We also predicted that responses at levels that were more similar in organization (e.g., enzymes vs. mitochondria) would be more similar than across distant scales (e.g., enzymes vs. whole animal metabolic rate). Additionally, we predicted that whole animal metabolic rates would be more likely to follow a standard TPC across measured temperature ranges, while rates at lower levels would more likely be exponential; this follows from the assumption that the thermal ranges sampled are likely to have been based on organismal

limits, rather the broader limits of processes at lower levels.

Materials and methods

Data collection

We searched the literature for publications where enzyme activities, mitochondrial respiration, and whole animal metabolic rates were measured across at least two temperatures. Search terms included combinations and iterations of keywords including "temperature," "thermal," "mitochondria," "respiration," "oxygen consumption," "plasticity," and "metabolism" (see Supplementary Table S1 for exact searches). We primarily searched publications indexed in the Web of Science database. Google Scholar was used in supplementary searches (examining the first 100 returned papers). We also used reference-based searching. Searches were performed during June and July 2019. Publications not indexed in databases or not publicly available, studies that were not published at the time of our searches, and those not written in English were likely missed.

To be included in our analyses, papers had to quantify mean and variance of a response measured at two or more temperatures across at least two of the three levels of biological organization. Only studies of ectothermic animals were included because ambient temperature controls body temperature in ectotherms, but not endotherms. In an effort to ensure consistency in methods such as animal husbandry when comparing across levels of biological organization, we only included papers where thermal response was measured at multiple levels of biological organization. For example, we did not compare whole animal metabolic rates from one publication to enzyme activities measured in a separate publication, even if the papers were published by the same research group and investigated the same species. We also required that acclimation and test temperatures were not confounded; animals had to be acclimated to one temperature and then tested across at least two temperatures. Metabolic rates had to be post-absorptive and taken under resting conditions (i.e., routine or standard metabolic rate) to be included. Although not explicitly an inclusion criterion, the temperatures investigated were often very similar or identical across levels of organization (i.e., metabolic rates measured at 10°C–20°C were not compared to enzyme activities at 20°C–30°C), which may be a limitation of these studies as thermal limits may differ across biological levels. The temperatures investigated also tended to be within the normal thermal range of the species.

Calculating effect sizes

For the publications included in our final dataset, the main data extracted were mean and variance of response (e.g., oxygen consumption or enzyme activity) at each temperature and associated sample sizes for those data. These data were taken directly from tables or text in the publication or were extracted from figures using WebPlotDigitizer version 4.2 (https://automeris.io/WebPlotDigitizer). One of us (ENKI) extracted all the data, but a random subset of 40% of the data were verified independently by another researcher (JCH). Several types of metadata were also extracted for each set of measurements, including taxonomic information for the species investigated, whether data were available for each level of organization, the acclimation temperature that was used prior to measuring responses across multiple temperatures, and the tissue that was used (for mitochondrial and enzymatic data). For most of the publications, multiple experiments were performed, including comparing among different species, populations, or mitotypes, using multiple acclimation treatments, comparing among multiple tissues, measuring multiple types of mitochondrial respiration (e.g., State 3 vs. State 4), or activities of multiple enzymes. These data were recorded for each set of measurements extracted from each publication.

The primary purpose of our study was to determine if thermal responses differed across levels of biological organization. Therefore, we developed an effect size metric, referred to here as "organizational disagreement" (OD) to quantify the disagreement in thermal responses when comparing measures at two different levels of biological organization (see Supplementary Information for a detailed statistical description). This essentially amounted to comparing the slope of the thermal response for each level of organization. Although slopes were assumed to be linear to allow common analysis of diverse shapes, we acknowledge that many responses were non-linear, which may have led to responses being quantified as more similar than they actually were. Importantly, because different scales were used for each of the responses, the relative change in response was used for the slope, not the absolute change in response (i.e., this method quantifies if rates doubled with some increase in temperature, not if they increased by a particular unit of measurement over that temperature). We coded the data so that OD would be positive if rates at lower organizational levels (e.g., enzyme activities) increased more with temperature than rates at higher organizational levels (e.g., metabolic rates), or negative if vice versa. We

therefore predicted positive OD based on our hypotheses. We used the delta method (Hoef 2012) along with the variances and sample sizes for each measurement to calculate the variance in OD for each comparison. The delta method has been used previously to estimate variance from thermal responses (Heine et al. 2019; Havird et al. 2020). Statistical details on calculating OD and the code used (as implemented in R, R Core Team 2016) can be found in the Supplementary Information.

In other words, for each publication, we extracted a series of thermal responses across levels of biological organization and then compared how much they differed using the OD effect size. For example, Fangue et al. (2009) measured two types of respiration in mitochondria isolated from liver tissue (S3 and S4 at 2°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C) along with whole animal metabolic rates (at 5, 10, 15, 20, 25, and 30°C) in a temperate and subtropical subspecies of *F. heteroclitus*. While mitochondrial respiration was measured in animals acclimated to 5°C, 15°C, and 25°C, whole animal metabolic rates were only quantified for animals acclimated to 5°C. Therefore, while 14 thermal responses were quantified, only four valid comparisons could be made: mitochondrial versus whole animal responses for two subspecies and two types of mitochondrial respiration. In this way, we never compared responses measured across different subspecies or populaitons, at different acclimation temperatures, or from different tissues.

Meta-analyses

We performed weighted, random-effects meta-analyses on the calculated OD metric and its variance using the metafor package in R (Viechtbauer 2010). In some analyses the "raw" value for OD was used to determine the directionality of the disagreement (e.g., if rates at lower organizational levels increased more with temperature than rates at higher organizational levels). However, we also performed meta-analyses on the absolute value of the organizational metric to answer the general question "How different are thermal responses at different levels of organization?" without considering the directionality of the disagreement. Publication was also included as a random effect in the main meta-analysis models to control for multiple comparisons being made from all publications. Publication bias in the data was assessed by visually inspecting a funnel plot and performing an Egger's regression (Egger et al. 1997), a trim-and-fill analysis (Duval and Tweedie 2000), and a fail-safe number analysis (Rosenthal 1979).

Meta-analyses were also performed with a variety of moderators to determine if certain variables affected OD. Acclimation temperature and absolute difference between acclimation temperature and the lowest test temperature were included as continuous moderators. Taxonomic class, tissue, enzyme identity, and type of mitochondrial respiration were included as categorical moderators in appropriate analyses. Finally, the type of comparison was included as a categorical moderator and included three categories: enzyme versus mitochondria, enzyme versus whole animal, and mitochondria versus whole animal.

Classifying the shape of thermal response curves

To determine if rates followed a stereotypical TPC (e.g., showing a peak value at an intermediate temperature), exponential increase with temperature as predicted by Arrhenius processes, or other patterns, each response was examined visually. For each response, the number of temperatures tested was recorded, along with whether rates generally increased, decreased, or did not change with temperature. The shape of each response was categorically classified as exponential, logarithmic, linear, logistic (i.e., "s-shaped"), "peak" (showing a clear increase to a peak followed by a clear decrease), or "valley" (decreasing to a clear minimal rate followed by a clear increase). Responses measured at three or more temperatures could be classified as any of these shapes (4 were required for s-shapred), while those measured at only two temperatures were classified as linear. Each response (means ± SEM) was visually inspected and scored blindly and independently by two of us (ENKI and JCH) and then examined for agreement. Although classifying responses using a quantitative best-fit approach would have been desirable, the lack of available raw data for most responses and the variability across the responses made this approach untenable. Differences in the proportion of response types between levels of organization were tested with Fisher's exact and chi-squared tests, and with a pairwise Fisher's test to determine if any particular types differed significantly by level.

Data availability

All data are available in the Supplementary Information or via FigShare (http://dx.doi.org/10.6084/m9.figshare.12047235). This includes the dataset used to calculate the OD effect size

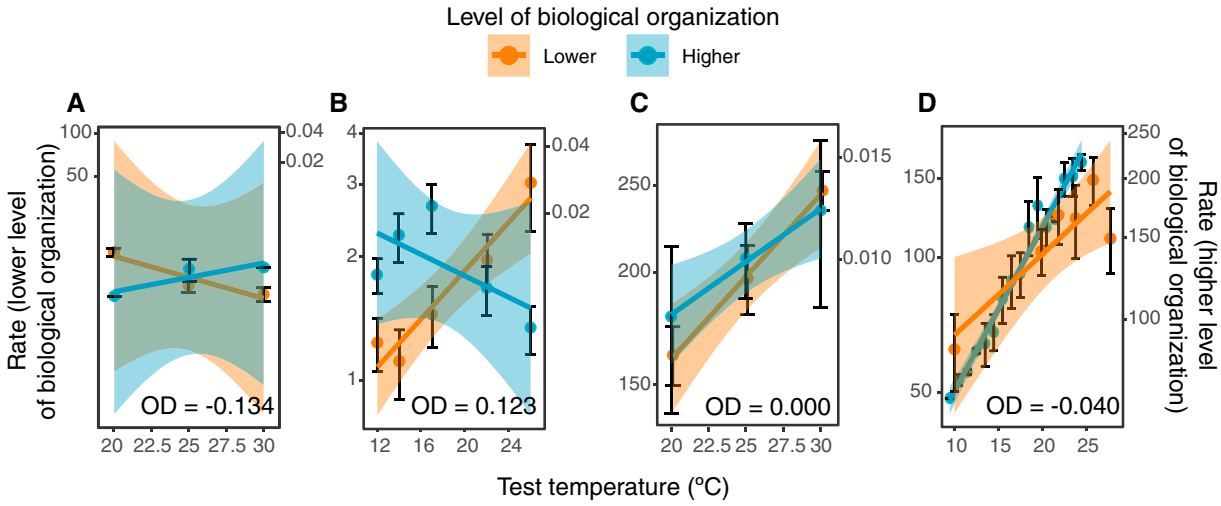

Fig. 3 Examples of the OD metric. (**A**) An extreme negative OD between cytochrome c oxidase activity (lower level) and S4 mitochondrial respiration (upper level) in *Crocodylus* (Glanville and Seebacher 2006). (**B**) An extreme positive OD between cytochrome c oxidase activity (lower level) and S3 mitochondrial respiration (upper level) in *Oreochromis* (Schnell and Seebacher 2008). (**C**) An example of limited OD between lactate dehydrogenase activity (lower level) and S3 mitochondrial respiration (upper level) in *Crocodylus* (Glanville and Seebacher 2006). (**D**) An example of "typical" OD in our dataset, shown between β-hydroxyacyl CoA dehydrogenase enzyme activity (lower level) and whole animal metabolic rate (upper level) in *Limnodynastes* (Rogers et al. 2007). Points are means, error bars show ±SEM, shading around lines of the best fit show 95% CI.

(Supplementary File S1), the dataset used to perform the meta-analyses for the 235 comparisons (Supplementary File S2), and the dataset for all 190 classified thermal responses (Supplementary File S3). Also included is the R code used to calculate effect sizes, perform the meta-analyses, and generate the figures (Supplementary File S4).

Results

OD varies widely

Over 1000 publications were screened during our searches and 13 publications representing 16 species from seven taxonomic classes met our inclusion criteria (Supplementary Fig. S1). Many publications quantified thermal responses at only one level of biological organization and were, therefore, excluded. Despite the limited number of publications included, 235 comparisons of thermal responses between different levels of biological organization were made. Rodnick et al. (2014) and Pörtner et al. (1999) had the lowest numbers of comparisons in our dataset (three each), while Rogers et al. afforded the most comparisons (90) owing to examining two acclimation treatments, two types of mitochondrial respiration, four enzyme activities, three tissues, and whole animal metabolic rate in the frog *Limnodynastes peronii* (Rogers et al. 2007). Given this, we reran the analyses after removing all data from Rogers et al. (2007) to ensure this single study was not solely responsible for the results. Qualitative results were similar regardless if this study was included or excluded.

OD was calculated for each of these 235 comparisons and varied from −0.134 to 0.123 (Fig. 3A and B). To put these values in context, simulated data where Q_{10} values were set to 100 at one level of organization and 0.01 at the other level produced an absolute organization disagreement value of 0.921. Q_{10} is a measure of the relative rate increase normalized to a 10° increase in temperature and Q_{10} values of 2–3 are considered normal (Schulte 2015). Therefore, OD values should conservatively vary from −1 (higher levels increase more rapidly with temperature) to 1 (lower levels increase more rapidly with temperature) for biological data of these sorts. We also recorded comparisons with very low levels of OD (Fig. 3C), where variance encompassed zero.

Thermal responses differ among levels of organization

Overall, there was noticeable disagreement in thermal responses among organizational levels, with absolute OD being significantly >0 overall ($OD = 0.038 \pm 0.003$, $P < 0.001$, Figs. 3D and 4A). When using raw OD to quantify the direction of disagreement, rates at higher levels of organization tended to increase more with temperature than rates at lower levels of organization (overall raw estimate $= -0.022 \pm 0.004$, $P < 0.001$, Fig. 4A). Q_{10} values supported this, with average Q_{10} for enzymes, mitochondria, and whole organisms being 1.55, 1.85,

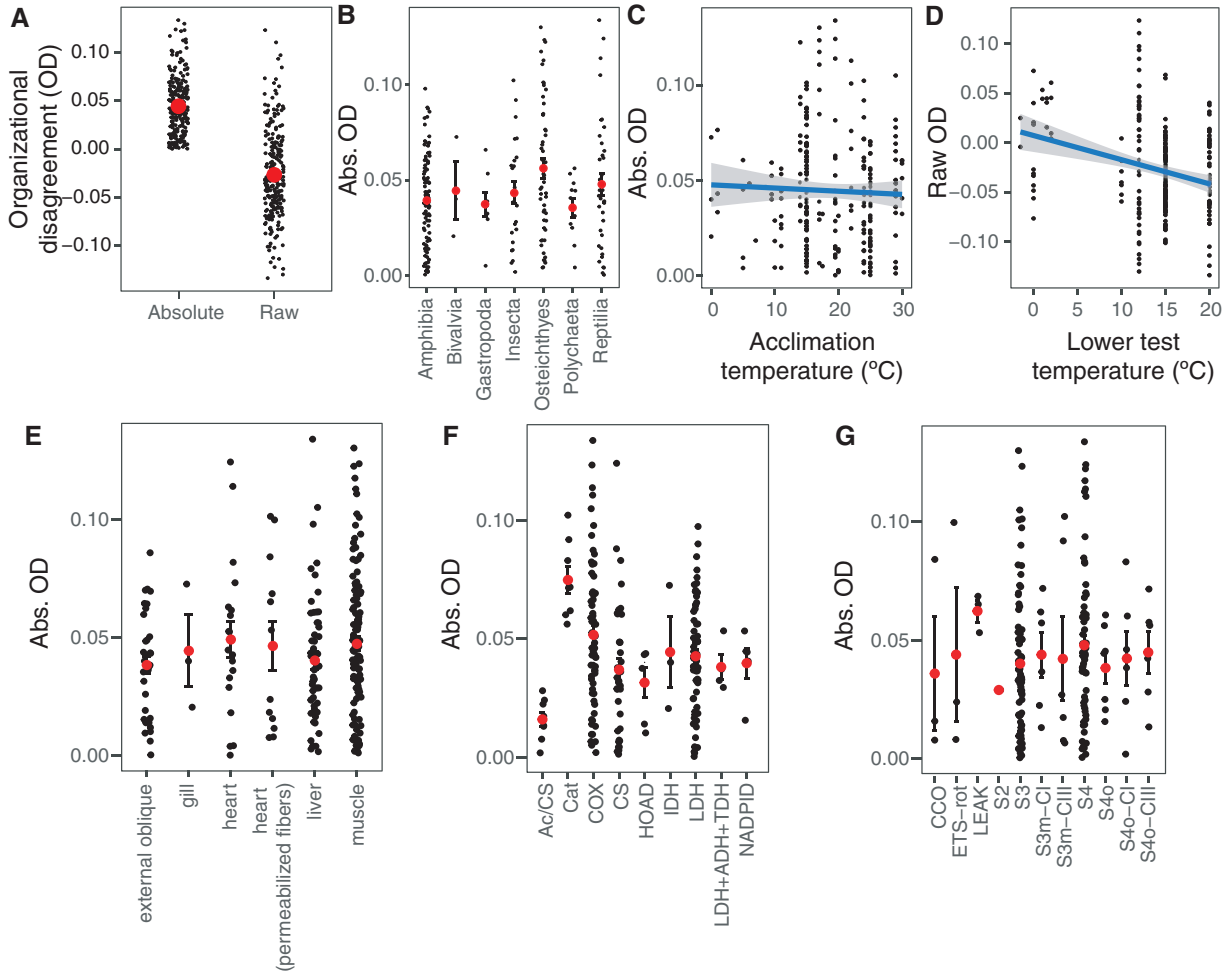

Fig. 4 OD differs only slightly among studies. (**A**) Absolute and raw OD for the entire dataset. (**B**) Absolute OD did not differ significantly among taxonomic classes examined. (**C**) Absolute OD did not change significantly with acclimation temperature. (**D**) Raw OD became slightly more negative as lower test temperature increased ($P < 0.001$). (**E**) Absolute OD did not differ significantly among tissues examined. (**F**) Absolute OD did differ significantly among enzymes examined ($P < 0.010$). (**G**) Absolute OD did not differ significantly among types of mitochondrial respiration examined. Red points show means, error bars show ±SEM, shading around lines of best fit show 95% CI. Ac, aconitase; CS, citrate synthase; Cat, catalase; COX/CCO, cytochrome c oxidase; HOAD, β-hydroxyacyl CoA dehydrogenase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; ADH, alcohol dehydrogenase; NADPID, NADP-dependent isocitrate dehydrogenase.

and 2.11, respectively. While heterogeneity between studies was low ($I^2 = 20.58\%$), there were significant differences among publications in "raw" OD (mean per study ranging from −0.055 to 0.025, $P < 0.001$). However, including publication as a random effect did not substantially alter results in any analysis.

Publication bias was investigated using a variety of methods, including generating funnel plots for both raw and absolute OD (Supplementary Fig. S2). Egger's tests for asymmetry in funnel plots suggested non-significant publication bias for raw OD ($P = 0.121$) and significant publication bias for absolute OD ($P = 0.033$). However, trim-and fill analyses did not substantially alter results of the meta-analyses and fail-safe numbers were large ($n = 2925$ and 9819 for raw and absolute OD, respectively), suggesting publication bias was a minor concern.

OD is similar among taxa, tissues, and acclimation treatments

Several moderators were investigated to determine when OD may be severe. Seven different classes of animals were included in our dataset, and while class was largely confounded with publication (e.g., no publications examined more than one class), there was not a significant difference in absolute OD among classes (Fig. 4B, ranging from 0.024 to 0.050, $P = 0.150$), such that in each class OD varied widely. Similarly, acclimation temperature or difference between acclimation and test temperature

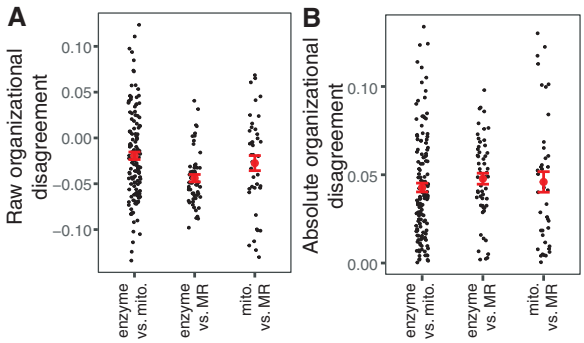

Fig. 5 OD is most extreme between the two most distant organizational levels: enzyme activities versus whole animal metabolic rates (MR) for both raw (**A**) and absolute (**B**) values of the OD metric. However, this was not statistically significant ($P = 0.070$ and $P = 0.678$, respectively). Red points show means, red error bars show ±SEM.

explained a non-significant portion of the variance in OD (Fig. 4C, although raw OD tended to increase with acclimation temperature, $P > 0.057$). There was a significant effect of lower test temperature on raw and absolute OD, such that as test temperatures increased, OD tended to increase (absolute OD increased by 0.013 per 10°C increase in test temperature, $P < 0.006$ for both; Fig. 4D). Different tissues used for mitochondrial isolation or enzyme activities showed similar absolute OD (Fig. 4E, ranging from 0.034 to 0.0466, $P = 0.857$). Enzyme activities did show different amounts of OD, with catalase showing particularly high OD ($P < 0.010$ for raw and absolute OD; max catalase = 0.076; min NADPID = 0.011; Fig. 4F). All types of mitochondrial respiration examined showed similar levels of absolute OD (Fig. 4G, ranging from 0.031 to 0.060, $P = 0.579$). Rates at the most dissimilar levels of biological organization (enzyme activities vs. whole animal metabolic rates) showed the most OD (Fig. 5), but comparison type was not a significant moderator for absolute (0.037–0.041; $P = 0.923$) or raw OD (−0.016 to −0.037, $P = 0.069$).

Thermal responses take a variety of shapes

From the 13 publications identified here, 189 unique thermal responses were extracted (Supplementary Figs. S3–S6). We classified the shape of each response, initially agreeing on the shape of 82% of the responses. The remaining 18% generally had points with high error, making classifications less straightforward. We discussed each of these ambiguous cases individually before settling on a response shape, and always favored "peak" shapes to be conservative.

On average, thermal responses were measured at four temperatures in our dataset (median = 3, range = 2–18), although more test temperatures tended to be used at higher levels of organization (Supplementary Fig. S7). For 31% of the thermal responses, rates were only measured at two temperatures, preventing any classification other than a linear response. Of these two-temperature responses, the vast majority (86%) showed increased rates with temperature, while the rest of the cases (8/58) showed no change in rates with increased temperatures. For the other 69% of responses where at least three temperatures were measured, rates overwhelmingly increased with temperature (86%), while no change or decreases with temperature were far less common (8% and 6%, respectively). For the 131 thermal responses where shape could be classified, linear responses were the most common (32%), although exponential responses were also common (29%). Logarithmic, "peak," "valley," and s-shaped responses were less common (17, 12, 5, and 5%, respectively; Fig. 6). The proportion of shapes initially appeared to differ significantly among organizational levels ($P = 0.064$ and 0.025 for Fisher's exact tests and chi-squared tests, respectively), with exponential responses at similar frequencies across levels, but "peak" responses being particularly common at the mitochondrial level. However, when pairwise Fisher's exact tests were performed, no significant enrichments for particular shapes at particular levels were found ($P > 0.290$ for all tests with FDR correction).

Discussion

Why is there OD in thermal responses?

Our main conclusion is that metabolic rates at different levels of biological organization respond to temperature differently (e.g., Fig. 3D), although our results should be interpreted cautiously due to the lack of complete datasets and the paucity of data points (averaging four test temperatures) for most thermal responses. OD is not inherently surprising, given that organismal phenotypes such as metabolic rate integrate processes playing out at many scales, while enzyme activities may be more directly influenced by strict thermodynamic laws.

Selection likely acts more strongly on processes at higher levels of organization because these levels are more tightly linked with fitness (Stearns et al. 1995) and because with greater complexity, there are more potential targets of selection. There are multiple ways to reach the same whole-organism thermal response based on different responses at lower levels of

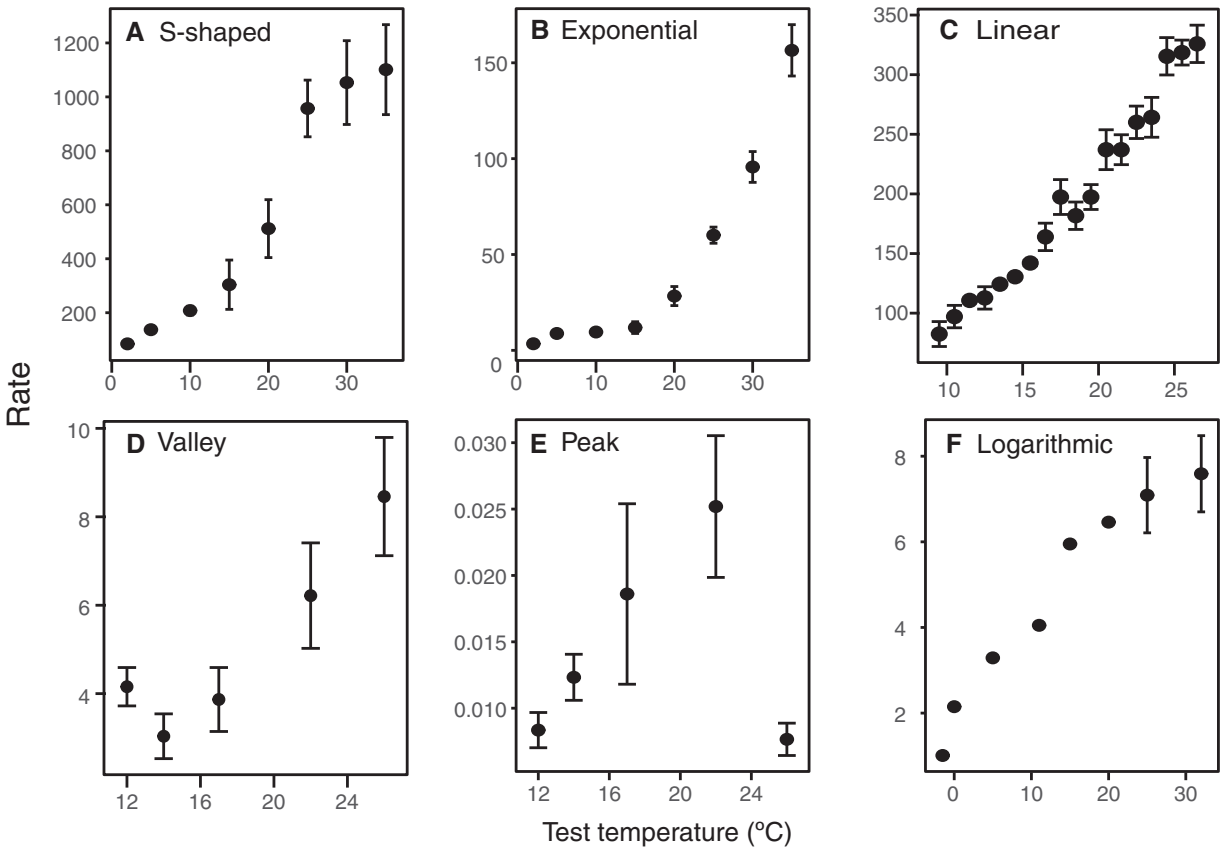

Fig. 6 Thermal responses can be classified into six different shapes. (**A**) s-shaped (S3 mitochondrial respiration in *Fundulus*, Fangue et al. 2009), (**B**) exponential (S4o mitochondrial respiration in *Fundulus*, Fangue et al. 2009), (**C**) linear (metabolic rate in *Salmo*, Penney et al. 2014), (**D**) "valley" (cytochrome c activity in *Oreochromis*, Schnell and Seebacher 2008), (**E**) "peak" (S3 mitochondrial respiration in *Oreochromis*, Schnell and Seebacher 2008), and (**F**) logarithmic (S3 mitochondrial respiration in *Arenicola*, Sommer and Pörtner 2002). Points are means, error bars show ±SEM.

organization. For instance, two organisms that differ in mitochondrial respiration rate might achieve the same whole-organism metabolic rate through varying mitochondrial densities. The OD metric described here provides a simple way to quantify differences in thermal responses among organizational levels and we suggest future studies make use of it to further explore the results described here. The biological consequences of OD may also be an arena for future work. Based on the range of OD values observed here, we suggest that absolute values greater than 0.1 be classified as "high" (likely indicating crossing reaction norms), absolute values of ~0.05 to be classified as "moderate," and absolute values <0.01 to be classified as "low" levels of OD.

Contrary to our expectations, OD was driven on average by greater relative rate increases at higher levels of organization. Assuming selection acts more strongly on organismal phenotypes such as metabolic rate, we expected whole animal metabolic rates to remain fairly constant with temperature compared to thermal responses at other levels. Both experimental limitations (discussed in the next section) and biology may explain this finding. Higher levels of organization would be expected to increase to a lesser degree based on the hypothesis of OCLTT, as increasing complexity constrains rates through processes not operating at lower levels (Pörtner 2010; Pörtner et al. 2017). However, in our dataset all measurements reflect acute temperature effects, also known as "passive plasticity" (Ghalambor et al. 2007; Kingsolver 2009; Schulte et al. 2011; Havird et al. 2020). If organisms had been acclimated to each temperature before being tested, whole organism rates may have increased less steeply due to acclimation, a form of "active plasticity" which involves physiological and transcriptional changes. Acclimation can occur at all levels of organization, but the capacity for acclimation should be greater at higher levels with more complex thermal responses (Burnell et al. 1991; Fangue et al. 2009). Similarly, rapid thermal transfers may cause a stress response resulting in highly elevated rates, which is more likely to manifest at higher compared to lower

levels of organization (Havird et al. 2020). Although acclimation and stress responses have not been explicitly tested across levels of biological organization, their differential effects on these levels may explain the unexpected results found here. For example, if stress responses were common in whole organisms and mitochondria, but relatively rare in enzymes, then negative OD would be expected.

Moderators, including taxon, acclimation temperature, and tissue, tended not to significantly predict OD, as evidenced by generally low heterogeneity among effects. One exception is enzyme identity, where catalase had a thermal response that disagreed significantly more with higher levels of organization than other enzymes. Catalase acts to decompose the reactive oxygen species hydrogen peroxide. While logically linked to mitochondrial respiration and metabolic rate, catalase is not found in the mitochondria of most cells (Bai et al. 1999; Bai and Cederbaum 2001; Schriner et al. 2005). This is in contrast to most of the other enzymes in our study, which are found in the mitochondria or directly participate in oxygen consumption (e.g., cytochrome c oxidase). Therefore, enzymes other than catalase may be more closely linked with, and show less OD with, the processes at higher levels examined here. This may also explain why the enzyme versus metabolic rates comparison tended to show the highest level of disagreement (although not statically different from other comparisons): levels or processes that are further removed may show more extreme disagreement compared with tightly linked processes.

While taxonomic or phylogenetic effects of OD likely exist, in our limited dataset they were largely confounded with the study and were not detected. The lack of an effect of acclimation temperature or the difference between acclimation temperature and lowest test temperature was particularly surprising, as acclimation is known to affect the shapes of organismal TPCs (Havird et al. 2020). Test temperature did significantly influence OD, with higher temperatures showing more OD. This may be because measurements at higher temperatures could encompass stress or dysfunction at some levels of organization, but not others. Many measurements in our dataset also increased in variance with temperature; higher temperatures may have caused more variability, which might explain the greater OD observed. Although we did find some evidence of publication bias in our dataset, we expect this is likely an artifact because the publications themselves did not address OD but were focused on thermal responses in general.

Stereotypical TPCs are rare in empirical data

We found that the reaction norms of the three biological rate processes we examined only rarely showed the predicted "peak" shape of a theoretical TPC (Schulte et al. 2011), with only 12% of responses fitting this shape. Rather, linear and exponential responses to temperature together comprised 61% of the data. One reason may be that TPCs are common but collected data may not sample the "falling leg" of the TPC (Fig. 6, Supplementary Figs. S3–S6; Dell et al. 2011). Supporting this, the falling leg only consisted of one or two points when "peak" shapes were apparent (e.g., Fig. 6E). This may also explain why rates tended to increase more dramatically with temperatures at higher levels of organization: negative OD could be driven by observing more of the falling legs at lower levels of organization. Another possibility is that responses with fewer numbers of data points may not have been easily classified as "peak." On average only four test temperatures were used in our dataset, possibly failing to capture the complete shape of a TPC. While more points were used at higher levels of organization (Supplementary Fig. S7), "typical" TPCs were rare across all levels.

One potential explanation for the lack of a falling leg in organismal-level responses is that enzymes and mitochondria can be more easily assayed at suboptimal high temperatures. Animals often experience mortality at the highest assay temperatures, and several of our studies cited mortality as a reason for low sample sizes or reducing the maximum test temperature for this level (Fangue et al. 2009; Penney et al. 2014). However, we did not observe significantly more instances of "peak" responses at lower levels of biological organization, especially at the enzymatic level. Therefore, the stereotypical idea of the TPC may be a largely theoretical concept when it comes to internal biological rates. Future studies should employ more gradual temperature increases at higher temperatures to limit mortality and sample the shape of the curve just before organismal failure (although confounding effects of acclimation may become problematic). They could also make use of Arrhenius break-point analysis (Sommer and Pörtner 2002; Hansen et al. 2017) to determine where the slope changes significantly and limit analyses to the rising leg for these comparisons (Dell et al. 2011).

The idea that peak rates of metabolism correspond to optimal temperatures or increased performance is controversial at best. While increased metabolic rates in ectotherms may allow greater levels of activity for

foraging and reproduction at lower temperatures (Huey et al. 2012), they may also represent increased costs associated with organismal maintenance (Pörtner 2010). While organismal phenotypes such as running speed or reproduction generally do follow TPCs and peak values are easily interpreted as increasing fitness (Schulte et al. 2011; Kellermann et al. 2019; Rezende and Bozinovic 2019), interpreting peaks in metabolic rates across levels of organization is less straightforward. A more insightful approach is to apply a TPC framework to aerobic scope, where maximal values may represent maximum energetic capacity available for survival and reproduction (Pörtner 2010; Pörtner et al. 2017; Gangloff and Telemeco 2018).

If rates at higher levels of organization were dependent upon pure thermo-kinetics at lower levels, then Arrhenius-like exponential increases should be by far the most common response. The preponderance of linear relationships may indicate that due to purely physiological limitation such as diffusion capabilities and compartmentalization, rates are constrained to increase more slowly (Clarke 2004; Clarke and Fraser 2004; Pörtner 2010). It may also indicate that acclimation can take place even at acute time scales. Physiological acclimation, such as vasodilation, changes in ventilation rate, and changes in tissue activity, can occur almost instantly, while significant transcriptional changes can occur within 30 min or less (e.g., Foster et al. 2015). Despite a common conflation of performance and metabolism in the literature, our results indicate that metabolic rate does not typically display the expected shape of a TPC. While performance metrics like aerobic scope may depend upon metabolic rate at lower temperatures, at higher temperatures they may decline for other reasons while metabolic rate is likely to still be increasing. Moreover, metrics such as running or swimming speed may depend on anaerobic respiration and may be unlinked to mitochondrial respiration or enzyme activities.

Future directions and importance in "genome to phenome" studies

One of the most surprising results of our searches was the general lack of studies where thermal responses were measured across multiple levels of organization. Many otherwise useful studies ($n = 103$) measured responses at only a single level of organization. Future studies should attempt to measure multiple organizational phenotypes at the same test temperatures to determine if the levels of disagreement shown here are representative of wider patterns, especially for marine invertebrates which were underrepresented in our dataset. Other meta-analyses of thermal responses across levels of organization have been performed, but did not control for species identity, acclimation temperature, or laboratory conditions (Dell et al. 2011; Rezende and Bozinovic 2019). One recent analysis of thermal responses at different organizational levels suggested that both lower-level subcellular processes and higher-level oxygen transport processes contribute to organismal thermal limits (Gangloff and Telemeco 2018), although many predictions stemming from these observations remain untested. Thus, while broad ecological patterns in thermal responses have been investigated, much remains to be done to explicitly link a response at one level of biological organization to another.

One consideration in genome to phenome studies is which phenotypes are of the most interest. While fitness is often the phenotype of most interest to evolutionary biologists, it is difficult to measure. Metabolic and other biological rates are often used as proxies for fitness. For example, when evaluating the responses of different populations or genotypes to climate change, metabolic rates are often considered (Cossins and Bowler 1987; Seebacher et al. 2015; Payne and Smith 2017; Rohr et al. 2018). The finding that thermal responses differ among levels of organization is important for theories that attempt to link physiology to ecology in the context of climate change (Brown et al. 2004; Pörtner 2010). This suggests researchers should consider which phenotypes are of interest in genome to phenome studies, as different phenotypes may be influenced differently by temperature and other environmental moderators.

Another consideration in genome to phenome studies is which genotypes are of the most interest. While most studies examine the effects of nuclear genetic variation, variation in the smaller mitochondrial genome may be especially important for predicting biological rates such as those measured here. Previous work has documented that different mitochondrial genotypes can interact with nuclear genetic variation and the environment to result in different phenotypes (i.e., $G \times G \times E$ interactions; Mossman et al. 2016; Camus et al. 2017; Mossman et al. 2017; Camus and Dowling 2018; Dobler et al. 2018). In our own dataset, one study examined *Drosophila simulans* populations that varied specifically in mitotype (Pichaud et al. 2010). One mitotype is distributed world-wide, while the other is found only in east Africa (Ballard 2003), leading to some speculation that the endemic mitotype may be relatively compromised. However, we found no

differences in OD between the two mitotypes. This illustrates how OD itself may be a phenotype of interest.

Explaining processes at higher levels of organization in light of lower levels is one of the most important lines of inquiry in biology and is an essential aspect of integrating from the genome to the phenome. Here we show, controlling for many other variables, that rate processes thought to be mechanistically integrated from lower to higher levels of organization respond to temperature differently. Aspects of the genome interact with the environment to create phenotypes that are manifested differently at different levels of organization (Fig. 2). At the level of the enzyme, relevant processes include not only the effects of temperature on the enzyme itself, but effects on chaperone proteins and proteases. The regulation and expression of these proteins are also impacted by temperature, an effect that is absent from the studies of isolated proteins in our dataset. At the level of the mitochondria even more effects are present, such as the impact of temperature on membrane condition, oxygen availability, and substrate concentration. There are also a great number of interacting parts in mitochondria (as mitochondria were once free-living organisms), including gene products from both the mitochondrial and nuclear genome. Finally, at the level of the organism, all effects at lower levels are present, as well as higher order effects such as blood-oxygen affinity, ventilation rate, temperature-sensing, and behavioral thermal responses. Examining whether selection acts differentially across these different levels should be a goal of future studies. For example, our dataset could be used to examine variance in rates at given temperatures across biological levels to evaluate the prediction that higher levels would show less variance owing to stronger selection. Studies like ours are important for clarifying the relationship between phenotypes across biological levels to begin to understand mechanisms of integration, common underlying genes, and phenomena that are relevant only at certain scales of organization.

Acknowledgments

We thank the organizers of the 2020 SICB symposium "Building Bridges from Genome to Phenome: Molecules, Methods and Models" for their invitation to contribute this work: K. G. Burnett, D. S. Durica, D. L. Mykles, and J. Stillman. We also thank D. H. Klinges, members of the Havird Lab, and participants in meta-analysis classes at the University of Texas and Auburn University for their comments on this work.

Funding

This study was supported by the University of Texas at Austin.

Supplementary data

Supplementary Data available at *ICB* online.

References

Arrhenius S. 1915. Quantitative laws in biochemical chemistry. London: Bell.

Bai J, Cederbaum AI. 2001. Mitochondrial catalase and oxidative injury. Biol Signal Recept 10:189–99.

Bai J, Rodriguez AM, Melendez JA, Cederbaum AI. 1999. Overexpression of catalase in cytosolic or mitochondrial compartment protects HepG2 cells against oxidative injury. J Biol Chem 274:26217–24.

Ballard JW. 2003. Sequential evolution of a symbiont inferred from the host: wolbachia and *Drosophila simulans*. Mol Biol Evol 21:428–42.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB. 2004. Toward a metabolic theory of ecology. Ecology 85:1771–89.

Burnell AM, Reaper C, Doherty J. 1991. The effect of acclimation temperature on enzyme activity in *Drosophila melanogaster*. Comp Biochem Physiol B 98:609–14.

Camus MF, Dowling DK. 2018. Mitochondrial genetic effects on reproductive success: signatures of positive intrasexual, but negative intersexual pleiotropy. Proc Biol Sci 285:20180187.

Camus MF, Wolff JN, Sgro CM, Dowling DK. 2017. Experimental support that natural selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian *Drosophila melanogaster*. Mol Biol Evol 34:2600–12.

Clarke A. 1993. Seasonal acclimatization and latitudinal compensation in metabolism - do they exist? Func Ecol 7:139–49.

Clarke A. 2004. Is there a universal temperature dependence of metabolism? Func Ecol 18:252–6.

Clarke A, Fraser K. 2004. Why does metabolism scale with temperature? Func Ecol 18:243–51.

Cossins AR, Bowler K. 1987. Temperature biology of animals. London/New York: Chapman and Hall.

Daan S, Masman D, Groenewold A. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. Am J Physiol 259:R333–40.

Dell AI, Pawar S, Savage VM. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. Proc Natl Acad Sci U S A 108:10591–6.

Dobler R, Dowling DK, Morrow EH, Reinhardt K. 2018. A systematic review and meta-analysis reveals pervasive effects of germline mitochondrial replacement on components of health. Hum Reprod Update 24:519–34.

Duarte CM. 2007. Marine ecology warms up to theory. Trends Ecol Evol 22:331–3.

Duval S, Tweedie R. 2000. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 56:455–63.

Egger M, Smith GD, Schneider M, Minder C. 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315:629–34.

Fangue NA, Richards JG, Schulte PM. 2009. Do mitochondrial properties explain intraspecific variation in thermal tolerance? J Exp Biol 212:514–22.

Foster NL, Lukowiak K, Henry TB. 2015. Time-related expression profiles for heat shock protein gene transcripts (HSP40, HSP70) in the central nervous system of *Lymnaea stagnalis* exposed to thermal stress. Commun Integr Biol 8:e1040954.

Gangloff EJ, Telemeco RS. 2018. High temperature, oxygen, and performance: insights from reptiles and amphibians. Integr Comp Biol 58:9–24.

Ghalambor CK, McKay JK, Carroll SP, Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Func Ecol 21:394–407.

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL. 2001. Effects of size and temperature on metabolic rate. Science 293:2248–51.

Glanville EJ, Seebacher F. 2006. Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. J Exp Biol 209:4869–77.

Grishkevich V, Yanai I. 2013. The genomic determinants of genotype x environment interactions in gene expression. Trends Genet 29:479–87.

Hansen AK, Byriel DB, Jensen MR, Steffensen JF, Svendsen MBS. 2017. Optimum temperature of a northern population of Arctic charr (*Salvelinus alpinus*) using heart rate Arrhenius breakpoint analysis. Polar Biol 40:1063–70.

Havird JC, Neuwald JL, Shah AA, Mauro A, Mashall CA, Ghalambor CK. 2020. Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q10 effects: why methodology matters. Funct Ecol 34:1015–28.

Heine KB, Abebe A, Wilson AE, Hood WR. 2019. Copepod respiration increases by 7% per degrees C increase in temperature: a meta-analysis. Limnol Oceanog Lett 4:53–61.

Hoef J. 2012. Who invented the delta method? Am Stat 66:124–7.

Huey RB, Kearney MR, Krockenberger A, Holtum JAM, Jess M, Williams SE. 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos Trans R Soc Lond B Biol Sci 367:1665–79.

Kellermann V, Chown SL, Schou MF, Aitkenhead I, Janion-Scheepers C, Clemson A, Scott MT, Sgro CM. 2019. Comparing thermal performance curves across traits: how consistent are they? J Exp Biol 222:jeb193433.

Kingsolver JG. 2009. The well-temperatured biologist. (American Society of Naturalists Presidential Address). Am Nat 174:755–68.

Mossman JA, Biancani LM, Zhu CT, Rand DM. 2016. Mitonuclear epistasis for development time and its modification by diet in *Drosophila*. Genetics 203:463–84.

Mossman JA, Tross JG, Jourjine NA, Li N, Wu ZJ, Rand DM. 2017. Mitonuclear interactions mediate transcriptional responses to hypoxia in *Drosophila*. Mol Biol Evol 34:447–66.

Payne NL, Smith JA. 2017. An alternative explanation for global trends in thermal tolerance. Ecol Lett 20:70–7.

Penney CM, Nash GW, Gamperl AK. 2014. Cardiorespiratory responses of seawater-acclimated adult Arctic char (*Salvelinus alpinus*) and Atlantic salmon (*Salmo salar*) to an acute temperature increase. Can J Fish Aquatic Sci 71:1096–105.

Pichaud N, Chatelain EH, Ballard JW, Tanguay R, Morrow G, Blier PU. 2010. Thermal sensitivity of mitochondrial metabolism in two distinct mitotypes of *Drosophila simulans*: evaluation of mitochondrial plasticity. J Exp Biol 213:1665–75.

Pörtner HO. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol A Mol Integr Physiol 132:739–61.

Pörtner HO. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J Exp Biol 213:881–93.

Pörtner HO, Bock C, Mark FC. 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J Exp Biol 220:2685–96.

Pörtner HO, Hardewig I, Peck LS. 1999. Mitochondrial function and critical temperature in the Antarctic bivalve, *Laternula elliptica*. Comp Biochem Physiol A Mol Integr Physiol 124:179–89.

Preedy VR, Paska L, Sugden PH, Schofield PS, Sugden MC. 1988. The effects of surgical stress and short-term fasting on protein synthesis in vivo in diverse tissues of the mature rat. Biochem J 250:179–88.

R Core Team T. 2016. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing (https://www.R-project.org/).

Rezende ELL, Bozinovic F. 2019. Thermal performance across levels of biological organization. Philos Trans R Soc Lond B Biol Sci 374:20180549.

Rodnick KJ, Gamperl AK, Nash GW, Syme DA. 2014. Temperature and sex dependent effects on cardiac mitochondrial metabolism in Atlantic cod (*Gadus morhua* L.). J Therm Biol 44:110–8.

Rogers KD, Thompson MB, Seebacher F. 2007. Beneficial acclimation: sex specific thermal acclimation of metabolic capacity in the striped marsh frog (*Limnodynastes peronii*). J Exp Biol 210:2932–8.

Rohr JR, Civitello DJ, Cohen JM, Roznik EA, Sinervo B, Dell AI. 2018. The complex drivers of thermal acclimation and breadth in ectotherms. Ecol Lett 21:1425–39.

Rosenthal R. 1979. The file drawer problem and tolerance for null results. Psych Bull 86:638–41.

Schnell AK, Seebacher F. 2008. Can phenotypic plasticity facilitate the geographic expansion of the tilapia *Oreochromis mossambicus*? Physiol Biochem Zool 81:733–42.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H. 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308:1909–11.

Schröer M, Saphorster J, Bock C, Pörtner HO. 2011. Oxygen and capacity limited thermal tolerance of the lugworm *Arenicola marina*: a seasonal comparison. J Exp Mar Biol Ecol 409:300–9.

Schulte PM. 2015. The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. J Exp Biol 218:1856–66.

Schulte PM, Healy TM, Fangue NA. 2011. Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr Comp Biol 51:691–702.

Seebacher F, White CR, Franklin CE. 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. Nat Clim Chang 5:61–6.

Somero GN. 1995. Proteins and temperature. Annu Rev Physiol 57:43–68.

Sommer AM, Portner HO. 2002. Metabolic cold adaptation in the lugworm *Arenicola marina*: comparison of a North Sea and a White Sea population. Mar Ecol Prog Ser 240:171–82.

Stearns SC, Kaiser M, Kawecki TJ. 1995. The differential genetic and environmental canalization of fitness components in *Drosophila melanogaster*. J Evol Biol 8:539–57.

Viechtbauer W. 2010. Conducting meta-analyses in R with the metafor package. J Stat Softw 36:1–48.

White CR, Alton LA, Frappell PB. 2012. Metabolic cold adaptation in fishes occurs at the level of whole animal, mitochondria and enzyme. Proc Biol Sci 279:1740–7.