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Comparing the temperature dependence of mitochondrial respiration among vertebrates

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ABSTRACT

Background: The metabolic theory of ecology (MTE) has often assumed that the temperature dependence of whole-organism respiration rate reflects only the biochemical kinetics of individual mitochondria summed across tissues. This assumption does not incorporate the many well-documented structural or functional changes in mitochondria that species may undergo to overcome, at least partially, thermodynamic constraints via acclimation. Yet, the validity of this basic assumption has scarcely been examined at broad scales.

Objective: We examine the temperature dependence of mitochondrial respiration *in vitro* across diverse vertebrates (fishes, mammals, birds, and amphibians) over a broad temperature range. We then compare this relationship to the temperature dependence of whole-organism respiration rate both within and across species.

Results: Despite differences in the quantity and composition of substrate, temperature alone explains the majority of variation in mitochondrial respiration rate across species. Also, respiration rate is similarly related to temperature at the level of mitochondria, within species at the level of whole organisms, and across species at the whole-organism level. These results contradict predictions based on the metabolic cold adaptation hypothesis, which rests on the assumption that adaptations allowing species to perform at higher levels in colder environments are of primary importance. Still, phylogeny and species identity explained between 7% and 16% of variance at the whole-organism level.

Conclusion: The biochemical kinetics of mitochondrial respiration in vertebrates is largely conserved across species and environments, irrespective of adaptation or acclimatization.

Keywords: metabolic rate, metabolic theory, respiration.

INTRODUCTION

The relationship between the temperature dependence of whole-organism respiration rate and that of individual mitochondria remains unclear (Bennett, 1980; Brown *et al.*, 2004; Angilletta and Angilletta, 2009), perhaps because of the possible influence of adaptation or acclimatization. One hypothesis, which we will refer to as the biochemical kinetics hypothesis, argues that at

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the level of both individual mitochondria and whole organisms, the temperature dependence of respiration rate is principally governed by thermodynamics. That is, biochemical kinetics explain most differences in rates of ATP synthesis in mitochondria, and thus differences in levels of aerobic respiration in whole organisms across temperatures (Bennett and Ruben, 1979; Bennett, 1980).

The biochemical kinetics hypothesis is foundational to the metabolic theory of ecology (MTE) (Brown *et al.*, 2004). The MTE argues that, after removing the effects of body size, the respiration rates of whole organisms and individual mitochondria should vary in the same way with temperature. More specifically, both are expected to be described by the Boltzmann-Arrhenius function, $e^{-E/kT}$, where E represents some average activation energy (0.6–0.7 eV), k is Boltzmann's constant (8.62×10^{-5} eV/K), and T is temperature in degrees Kelvin (Gillooly *et al.*, 2001, 2005). However, implicit in this model are three largely untested simplifying assumptions:

1. mitochondria from all species are functionally equivalent;
2. the range 0.6–0.7 eV reflects the average activation energy of biochemical reactions in mitochondria; and
3. the effects of adaptation and/or acclimatization are secondary across a broad temperature range.

Perhaps more than any other assumption, the potential effects of adaptation and/or acclimatization have raised questions about whether the temperature dependence of whole-organism respiration can be modelled based on the biochemical kinetics of individual mitochondria. Many features of mitochondria may change across gradients in body temperature to allow species to overcome simple thermodynamic constraints, at least partially (Clarke and Fraser, 2004), including changes in the stability and conformation of mitochondrial enzyme proteins, changes in the membrane area or composition of mitochondria, and/or changes in mitochondrial density (Johnston *et al.*, 1998; Moyes, 2003; Clarke and Fraser, 2004; Guderley *et al.*, 2005; Schulte, 2015). Such adaptations might allow species to perform at higher levels in colder environments, as proposed by the metabolic cold adaptation hypothesis (Holeton, 1974; Clarke, 1980; Addo Bediako *et al.*, 2002). Furthermore, these adaptations predict that the temperature dependence of respiration rate at the whole-organism level should be steeper within species than across species, and, to some degree, that mitochondria from diverse species are not functionally equivalent. However, the extent to which acclimatization and/or adaptation challenges the biochemical kinetics hypothesis has been hard to discern, in part because the temperature dependence of mitochondrial respiration rate has not been examined across species at a scale comparable to that of whole-organism respiration rate.

Here, we assess the effects of temperature on the respiration rates of isolated mitochondria across diverse vertebrates (amphibians, fishes, mammals, and birds) from a range of environments. We then compare this relationship to the temperature dependence of whole-organism respiration rate both within and across species. The biochemical kinetics hypothesis invoked by Gillooly *et al.* (2001) and MTE predict the same temperature dependence for all three relationships. Conversely, if adaptation along temperature gradients is of primary importance, the temperature dependence of whole-organism respiration rate across species should be weaker than the other two relationships (Clarke and Fraser, 2004).

METHODS

Data collection

To examine the temperature dependence of mitochondrial respiration, we compiled published data on oxygen consumption rates across a range of temperatures for intact mitochondria from fresh tissue of diverse vertebrates. We measured all mitochondria under 'state 3' conditions.

The dataset included 93 total measures from 9 fish species, 7 mammal species, 13 bird species, and 1 amphibian species (evolutionary-ecology.com/data/3184Appendix.pdf, Table S1). The dataset, which we compiled to represent vertebrate diversity broadly, was restricted to measures from liver tissue to control for potential effects of tissue type (Moyes, 2003; Hulbert *et al.*, 2006). Studies used different combinations of three substrates (succinate, pyruvate, and/or malate) at varying concentrations to fuel mitochondrial respiration. But, given the scale of our analysis, we could not control for possible effects of difference in substrate.

We restricted measures of oxygen consumption rate at different temperatures to those up to and including optimal temperatures (i.e. temperature at maximal consumption rate), since the Boltzmann-Arrhenius function does not apply beyond these temperatures (Savage *et al.*, 2004). Mitochondrial respiration measures were converted as necessary to standard units of nmol O/min/mg mitochondrial protein (Gnaiger, 2014).

To quantify the temperature dependence of resting oxygen consumption rates in vertebrates, we relied primarily on data from White *et al.* (2006), and then supplemented these data with data from Clarke and Johnston (1999) to provide broader taxonomic representation for fishes. (Appendix Table S2 has the data.) For ectotherms, oxygen consumption rates were measured for resting individuals held at constant environmental temperatures. For endotherms, direct measures of body temperature were used to assess the temperature dependence of resting oxygen consumption. For species with multiple measures at a given temperature, we included only data from the largest individual(s), thus restricting analyses to subadult or adult individuals because respiratory systems may change during ontogeny (Post and Lee, 1996; Maina and West, 2005). Species not present in the vertebrate phylogeny used here [$n = 50$; Open Tree of Life (Hinchliff *et al.*, 2015)] were not included in analyses (see Table S3 for a list of these taxa).

We used generalized linear mixed-models (GLMMs) to assess the temperature dependence of mitochondrial respiration rate across vertebrates, and to assess the temperature dependence of whole-organism respiration rate for each vertebrate class. We analysed whole-organism respiration rates for mammals and birds together to provide a broader range of temperatures, and to remain consistent with previous analyses of these groups (Gillooly *et al.*, 2001). In all cases, we determined the temperature dependence by fitting linear models of the form: $\ln(\textit{respiration rate}) \sim E \times (1/kT) + \text{intercept}$. For whole-organism respiration rate, we included $\ln(\textit{body mass})$ as a fixed effect to control for effects of body mass. We did not need to do that for the analysis of mitochondrial respiration, since $\ln(\textit{body mass})$ was not a significant correlate in this case ($P > 0.05$).

We fitted models using a Bayesian Monte Carlo Markov Chain (MCMC) approach (Hadfield and Nakagawa, 2010) implemented with the R package 'MCMCglmm' (Hadfield, 2010). For each model, we included phylogenetic covariance matrices as random effects. We took phylogenetic relationships from the Open Tree of Life (Hinchliff *et al.*, 2015) using the R package

'rotl' (Michonneau *et al.*, 2016) (phylogenies given in the Appendix, Supplementary Dataset 2), and branch lengths were estimated with Grafen's method using the R package 'ape' (Grafen, 1989; Paradis *et al.*, 2004). For datasets that included multiple points per species (i.e. the datasets for mitochondrial respiration and for ectotherms at the whole-organism level), we also included species identity as a random variable (de Villemereuil and Nakagawa, 2014). We compared the variance explained by fixed effects (temperature, body mass) to the variance explained by both fixed (temperature, body mass) and random (phylogeny, species identity) effects using marginal and conditional R^2 values, respectively (Nakagawa and Schielzeth, 2013). We used partial residuals of the phylogenetic mixed models to plot effects of temperature on respiration rate.

We used linear models to evaluate the temperature dependence of whole-organism respiration rate within species of ectotherms. We fitted models using ordinary least squares regression to species-level data that ranged in temperature by ≥15°C, and we included ln(*body mass*) as a predictor variable in cases where body mass was a significant predictor ($P < 0.05$). We summarized variability in E with a histogram, and by calculating the mean and standard deviation for each taxonomic group (i.e. fishes, reptiles, and amphibians). We excluded species with a temperature dependence not significantly different from 0 (i.e. $P > 0.05$) from this analysis. Endothermic species were also excluded from these intraspecific analyses, since body temperatures are relatively constant.

RESULTS

Temperature explained most of the variation in respiration rates of isolated mitochondria, after we accounted for phylogeny, and despite any differences in substrate (marginal $R^2 = 0.64$; Fig. 1, Table 1). The full model, including phylogeny and species identity as random effects, explained approximately 95% of the variance in mitochondrial respiration rate (conditional R^2; Table 1). Thus, phylogeny and species identity explained ~31% of the variation. The exponential increase in mitochondrial respiration with temperature that we observed was also consistent with predictions from MTE ($E = -0.61$; $n = 93$; 95%CI: −0.66 to −0.56; Fig. 1). This corresponds to a Q_{10} of 2.58 at 0°C, and 2.06 at 40°C, given that $Q_{10} \approx e^{(10E/kT\wedge 2)}$ (Gillooly *et al.*, 2001).

The temperature dependence of mitochondrial respiration largely matched the interspecific relationship of whole-organism respiration rate to temperature (Fig. 2A–D; Table 1). For whole-organism respiration rate across species, the phylogenetic mixed models estimated $E = -0.58$ for fishes (95% CI: –0.64 to –0.52, $n = 376$), $E = -0.65$ for reptiles (95% CI: −0.69 to −0.60, $n = 428$), and $E = -0.61$ for mammals and birds (95% CI: −0.75 to −0.46, $n = 524$), which were statistically indistinguishable from the value of $E = -0.61$ for mitochondria. But for amphibians, the observed temperature dependence of whole-organism respiration across species was significantly weaker ($E = -0.53$; 95% CI: –0.58 to −0.48, $n = 445$). For all groups, temperature and body size explained most of the variance in respiration rates as measured by the marginal R^2 (fishes: 0.80; reptiles: 0.85; amphibians: 0.84; mammals/birds: 0.90). Phylogeny and species identity accounted for less variance, ranging from ~7% in mammals/birds to ~16% in fishes.

The temperature dependence of whole-organism respiration within species was also similar to that of mitochondrial respiration (Fig. 3). The mean activation energy for fishes ($E = -0.62$, SD = 0.10), reptiles ($E = -0.68$, SD = 0.19), and amphibians ($E = -0.62$, SD = 0.20) were all close to that of mitochondria ($E = -0.61$). For fishes and reptiles,

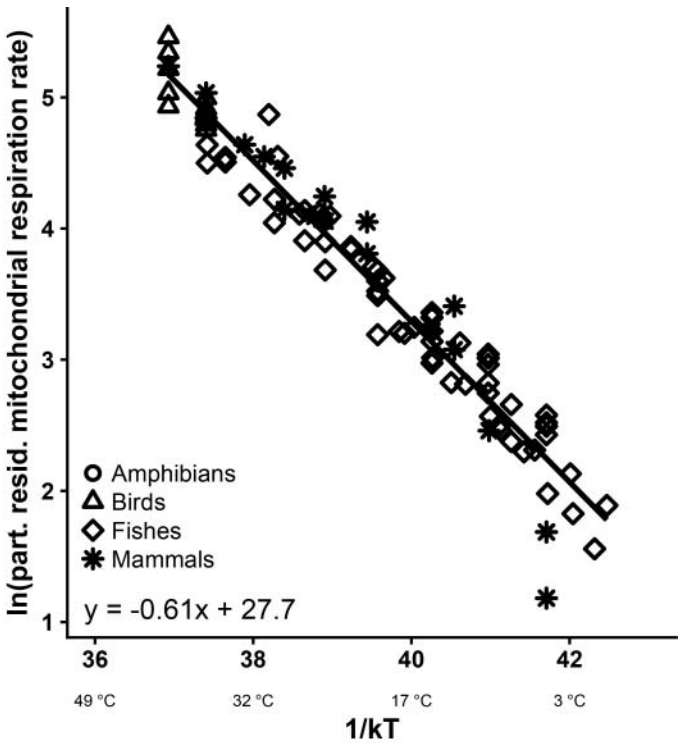

Fig. 1. Effect of temperature on the respiration rates of isolated mitochondria in vertebrates (units: nmol O/min/mg mitochondrial protein). The ln(*respiration rate*) is plotted against inverse absolute temperature ($1/kT$) as partial residuals from a phylogenetic mixed model (Table 1). The Celsius scale is rounded to the nearest whole number.

Table 1. Statistics from mixed models that describe the temperature dependence of vertebrate respiration in mitochondria (nmol O/min/mg mitochondrial protein) and whole organisms (mL O_2/hour)

	N	*E*	ln(*body mass*)	Intercept	R^2_{marg}	R^2_{cond}
Mitochondria	93	−0.61 (−0.66, −0.56)	N/A	27.7 (25.7, 29.8)	0.64	0.95
Whole organisms						
Fishes	376	−0.58 (−0.64, −0.52)	0.91 (0.87, 0.95)	20.4 (17.9, 22.7)	0.80	0.96
Reptiles	428	−0.65 (−0.69, −0.60)	0.82 (0.77, 0.89)	23.0 (21.3, 24.9)	0.85	0.97
Amphibians	445	−0.53 (−0.58, −0.48)	0.80 (0.76, 0.84)	18.5 (16.6, 20.6)	0.84	0.93
Mammals/Birds	524	−0.61 (−0.75, −0.46)	0.70 (0.68, 0.72)	24.1 (18.3, 29.4)	0.90	0.97

Note: The variance explained only by fixed effects, and by both fixed and random effects, is described in terms of marginal and conditional R^2 values (i.e. R^2_{marg} and R^2_{cond}), respectively. All parameter estimates are statistically significant ($P < 0.001$).

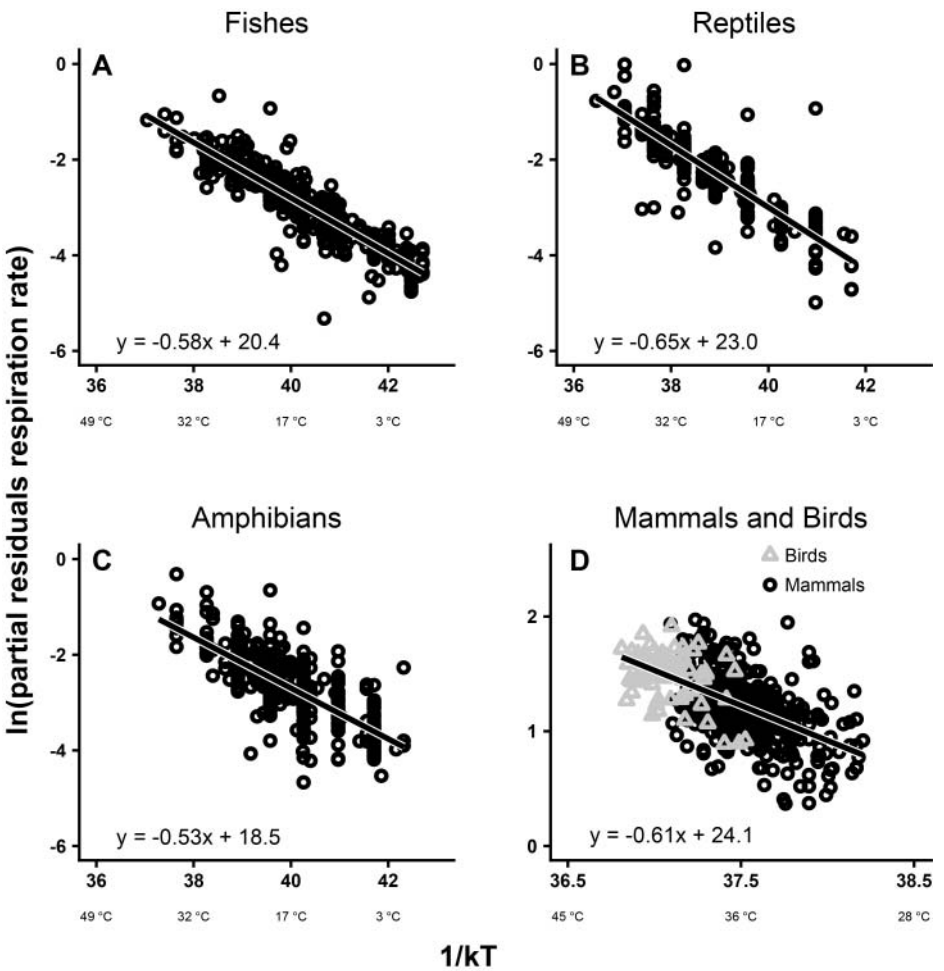

Fig. 2. Effect of temperature on whole-organism respiration rate (in mL O_2/hour) in (A) fishes, (B) reptiles, (C) amphibians, and (D) endotherms (birds and mammals). The ln(*respiration rate*) is plotted against inverse absolute temperature ($1/kT$) as partial residuals from a phylogenetic mixed model for each class of vertebrates (Table 1). The Celsius scale is rounded to the nearest whole number.

the observed temperature dependence within species was similar to the temperature dependence that we observed across species (fishes: $E = -0.62$ vs. -0.58; reptiles: $E = -0.68$ vs. -0.65), whereas for amphibians E was greater within species than across species ($E = -0.62$ vs. -0.53).

DISCUSSION

Our results show that, at the level of mitochondria, the biochemical kinetics of respiration is highly conserved across diverse vertebrate species. This suggests that adaptation or acclimatization has relatively little impact on respiration across broad gradients in temperature at the level of individual mitochondria. As such, our results provide some support

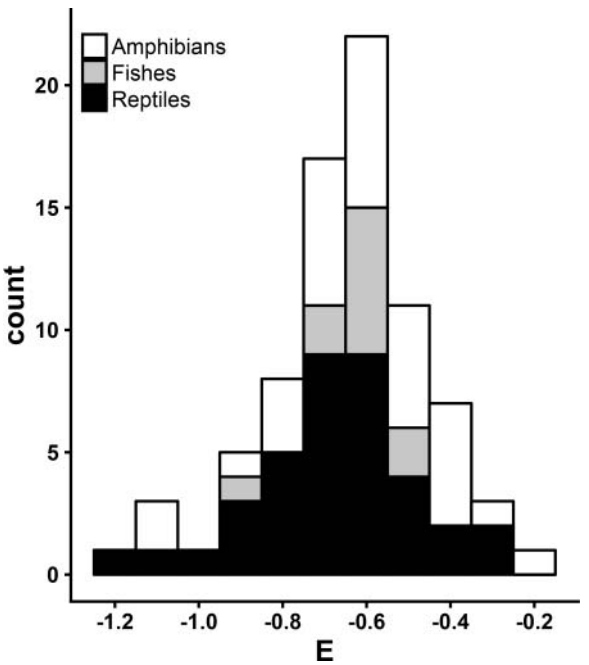

Fig. 3. Histogram summarizing intraspecific variation in the temperature dependence of whole-organism respiration rate for ectothermic vertebrates measured at a temperature range of ≥15°C. The average activation energy, E, corresponds to the slope of the relationship between respiration rate and inverse absolute temperature ($1/kT$; see Fig. 1).

for the assumption of Gillooly *et al.* (2001) and the metabolic theory of ecology (Brown *et al.*, 2004) that the temperature dependence of whole-organism respiration rate reflects the biochemical kinetics of individual mitochondria.

This appears to be the case despite the fact that not all oxygen consumed by organisms is for mitochondria, and that not all ATP synthesis is coupled to mitochondrial oxygen consumption. For mammals, Rolfe and Brown (1997) estimate that no more than 90% of oxygen consumption is mitochondrial, and 80% of that is linked to ATP synthesis. Still, one must keep in mind that the explanatory power of temperature as an independent variable relative to other variables will likely depend on the scope of temperature variation. At the broad temperature range we examine, and with the effect of temperature being exponential, we expect temperature to be of primary importance. The effects of other relevant variables on mitochondrial respiration (e.g. substrate type or concentration) should be of secondary importance. Note, too, that data for mitochondrial respiration were dominated by bird, fish, and mammal species. Data for amphibians and reptiles were rare or non-existent (Fig. 1). Nonetheless, we observe no obvious differences in mitochondrial respiration across classes of vertebrates. Thus, at least in this one tissue (liver), mitochondria do appear to be approximately equivalent in terms of function.

The temperature dependence of mitochondrial respiration rate, whole-organism respiration rate within species, and whole-organism respiration rate across species, were similar to each other and consistent with the predictions of MTE. With the exception of

amphibians, whole-organism respiration rates for each class of vertebrates showed a temperature dependence that was statistically indistinguishable both from the value of $E = 0.6–0.7$ eV predicted by Gillooly *et al.* (2001), and from the value of $E = 0.61$ observed for mitochondria (Fig. 1). Still, our results may provide some support for the metabolic cold adaptation hypothesis in the case of fishes and amphibians. For fishes, our phylogenetic mixed model yielded a much steeper temperature dependence ($E = 0.58$) than previous non-phylogenetic analyses of fish respiration [$E = \sim 0.4$ (Clarke and Johnston, 1999; White *et al.*, 2006)]. This observation is consistent with previous work in fishes showing that residual variation in the relationship of respiration rate to temperature is partially explained by differences in taxonomy (Clarke and Johnston, 1999), which in turn is associated with differences in mitochondrial densities and/or enzyme activities (Johnston *et al.*, 1998; Guderley, 2004; White *et al.*, 2011). For amphibians, the average value of E within species (0.62) was steeper than the interspecific relationship ($E = 0.53$), consistent with expectations from the metabolic cold adaptation hypothesis. However, the strongest evidence for metabolic cold adaptation continues to be research in fishes dominated by polar species (White *et al.*, 2011).

The effects of temperature on mitochondrial respiration rate shown here may also be relevant beyond direct considerations of respiration rate. Increased mitochondrial respiration rates at higher temperatures may lead to higher rates of mitochondrial turnover and decay (Fletcher and Sanadi, 1961), and higher rates of ageing and disease occurrence associated with mitochondrial function (Shigenaga *et al.*, 1994; Lin and Beal, 2006). A better understanding of these possible links will require more broad-scale studies of mitochondrial structure and function across species.

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DATA AVAILABILITY

All data used in this study are available at evolutionary-ecology.com/data/3184Appendix.pdf.

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