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Testing the metabolic homeostasis hypothesis in amphibians

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A number of hypotheses about compensatory mechanisms that allow ectothermic animals to cope with the latitudinal decrease in ambient temperature (T_A) have been proposed during the last century. One of these hypotheses, the 'metabolic homeostasis' hypothesis (MHH), states that species should show the highest thermal sensitivity of the metabolic rate ($Q_{10\text{-SMR}}$) at the colder end of the range of T_As they usually experience in nature. This way, species should be able to minimize maintenance costs during the colder hours of the day, but quickly take advantage of increases in T_A during the warmer parts of the day. Here, we created a dataset that includes $Q_{10\text{-SMR}}$ values for 58 amphibian species, assessed at four thermal ranges, to evaluate three predictions derived from the MHH. In line with this hypothesis, we found that: (i) $Q_{10\text{-SMR}}$ values tended to be positively correlated with latitude when measured at lower T_As, but negative correlated with latitude when measured at higher T_As, (ii) $Q_{10\text{-SMR}}$ measured at lower T_As were higher in temperate species, whereas $Q_{10\text{-SMR}}$ measured at higher T_As were higher in tropical species, and (iii) the experimental T_A at which $Q_{10\text{-SMR}}$ was maximal for each species decreased with latitude. This is the first study to our knowledge showing that the relationship between $Q_{10\text{-SMR}}$ and latitude in ectotherms changes with the T_A at which $Q_{10\text{-SMR}}$ is assessed, as predicted from an adaptive hypothesis.

This article is part of the theme issue 'Physiological diversity, biodiversity patterns and global climate change: testing key hypotheses involving temperature and oxygen'.

1. Introduction

Environmental temperature (air temperature, T_A) represents one of the most crucial abiotic factors influencing the evolutionary trajectories of physiological and ecological traits of most terrestrial organisms on Earth [1]. Sensitivity to T_A is particularly high in terrestrial ectotherms whose metabolic functions directly depend on the access to external sources of heat [2]. Therefore, the performance of most basic functions with a direct contribution to fitness (e.g. physiology, growth, reproduction) in a terrestrial ectotherm will respond to T_A changes, a plastic phenotypic response known as thermal reaction norm. Among the traits that are directly affected by changes in temperature, the standard metabolic rate (SMR) has been suggested as a good indicator of the general costs of body maintenance [3,4]. Thus, SMR has been used to determine whether ectothermic animals modulate their maintenance costs in response to changes in environmental conditions [3,5]. In addition, the thermal response in SMR can act as a proxy of the overall rate of response of an organism, since temperature effects on SMR usually positively covary with the thermal responses in growth and reproductive rates (see [6]).

A number of hypotheses based on the association between T_A and physiological variables, like metabolic rates and thermal tolerance ranges, have been proposed to explain geographical patterns of variation in distribution ranges [7,8]. In particular, the lower temperatures, shorter growing seasons and extreme seasonality of higher latitudes may restrict the amount of energy an ectothermic individual is able to expend, and this could result in a decrease in SMR [9], growth rate [10], and ultimately individual fitness and population growth [11]. As such, two non-mutually exclusive hypotheses have been proposed to explain the compensatory mechanisms that would prevent such a reduction in fitness at higher latitudes. The first one is the 'metabolic cold adaptation' hypothesis (MCA), which predicts that at similar T_A, the metabolic rate exhibited by individuals from species or populations experiencing colder climates is greater than that of their warm-climate relatives [12–16]. The second one is the 'climatic variability' hypothesis (CVH), which posits that as the range of climatic fluctuation experienced by terrestrial animals increases with latitude, individuals at higher latitudes would require a broader range of tolerances or greater acclimation abilities (phenotypic plasticity) to persist at a site [17–20].

A third, much less assessed hypothesis about compensatory mechanisms, is the 'metabolic homeostasis' hypothesis (MHH; [21]). The MHH proposes that T_A has shaped metabolic curves (via natural selection)—by adjusting the thermal dependence of different cellular and biochemical processes—causing the thermal sensitivity of the standard metabolic rate ($Q_{10\text{-SMR}}$, i.e. the change in SMR due to an increase of temperature by $10°C$) to differ among species inhabiting different zones of the globe. Specifically, this hypothesis states that $Q_{10\text{-SMR}}$ should rapidly increase towards the colder end of the thermal range experienced in nature, and reach a plateau at those temperatures where animals are maximally active in the field. In this way, species should be able to minimize maintenance costs during the colder hours of the day (when they remain inactive), but quickly take advantage of increases in T_A during the warmer parts of the day (figure 1a). A corollary of the MHH is that $Q_{10\text{-SMR}}$ should increase with latitude when measured at lower temperatures, but decrease with latitude if measured at higher temperatures (figure 1b). Even though some empirical support for this result already exists (e.g. [22,23]), no study has assessed the MHH at a large spatial scale and in an explicit comparative framework. Therefore, the aim of the present study was to evaluate the relationship between thermal sensitivity of the standard metabolic rate (measured at four different T_A ranges) and geographical latitude, at a global scale in a phylogenetic context. Specifically, we tested three predictions derived from the MHH: (i) $Q_{10\text{-SMR}}$ values should be positively correlated with latitude when measured at lower T_As, but negatively correlated with latitude when measured at higher T_As; (ii) $Q_{10\text{-SMR}}$ values estimated at lower T_As should be higher for temperate species than for tropical species, while the contrary should hold true for $Q_{10\text{-SMR}}$ values estimated at higher T_As; and (iii) there should be a negative association between the experimental temperature at which $Q_{10\text{-SMR}}$ reaches the highest value for each species and geographical latitude (i.e. the greatest thermal response should be observed at lower experimental temperatures as latitude increases). We focused our comparative study on the
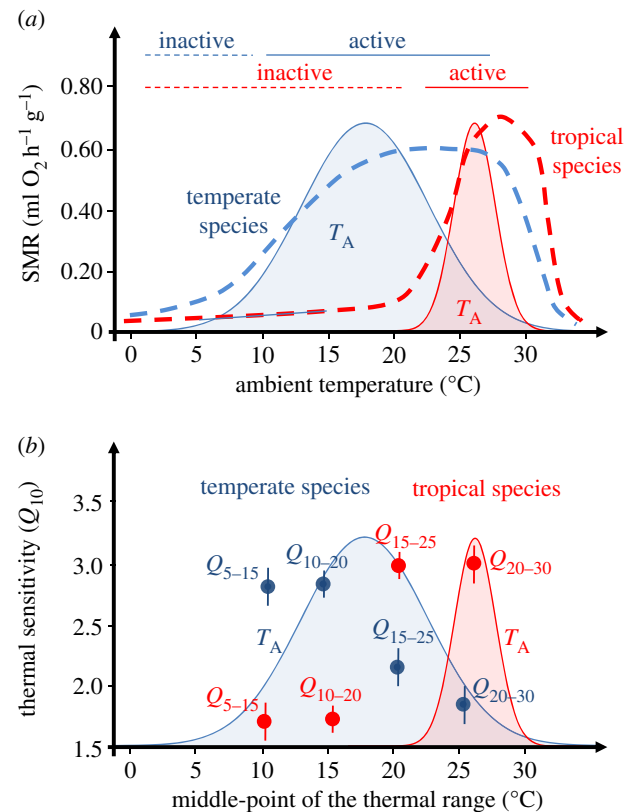

Figure 1. (a) Relationship between standard metabolic rate (SMR) and ambient temperature (T_A) for a hypothetical temperate species (bold blue dashed line) and for a hypothetical tropical species (bold red dashed line). (b) Thermal sensitivity (Q_{10}) values as a function of the experimental thermal range for each hypothetical species; these results were derived from the SMR curves depicted in (a). The period of activity/inactivity for each species is marked with a horizontal continuous/dashed line. In both panels a hypothetical distribution of T_A for a temperate (blue bell) and a tropical (red bell) habitat is represented by a Gaussian curve.

vertebrate class Amphibia for both theoretical and practical reasons. First, amphibians exhibit an extraordinary biological diversity and evolutionary novelty, having a pivotal role between terrestrial and aquatic communities [24–26]. Second, amphibians are ectothermic vertebrates in which the adult phase of the life cycle, mostly occurs in terrestrial habitats (or small water bodies). In this sense, we emphasize that our predictions would not hold for species inhabiting large masses of water (e.g. oceans, large lakes) in which daily variability in temperature is relatively narrow ([2] and references therein). Third, a comprehensive study compiling all the data published to date on SMR for this taxonomic group has been recently published [27]. Thus, to some extent, we are using amphibians as a first test case of general ideas for all terrestrial ectotherms.

2. Material and methods

(a) Database description

Based on the review published in [27], we compiled studies reporting data on body mass, collection site and VO_2 consumption for at least two of the following ambient temperatures: 5, 10, 15, 20, 25 and 30°C (electronic supplementary material, table S1). After a preliminary search, we decided to exclude studies published before 1970 to reduce the effect of confounding factors associated with methodological (e.g. estimation of VO_2 in animals fitted with masks) and statistical (e.g. calculation of
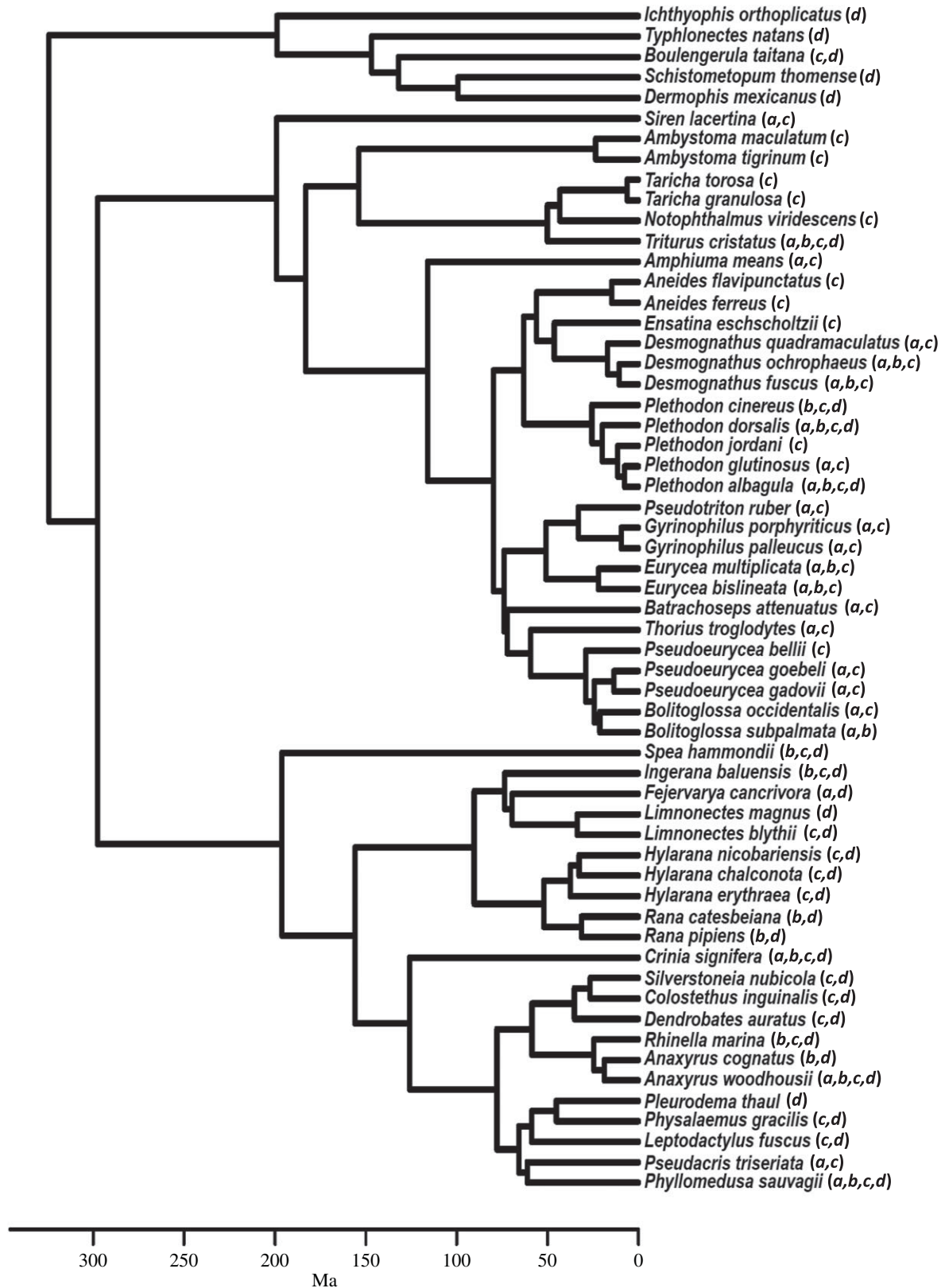
Figure 2. Phylogenetic trees used in phylogenetic analysis. The particular species that were included in each comparison (i.e. thermal range) are indicated with letters: (a) 5–15°C, (b) 10–20°C, (c) 15–25°C, (d) 20–30°C. Ma = million years ago.

$Q_{10\text{-SMR}}$ from eye-fitted curves) issues. This way, all the studies included in our dataset measured SMR by using metabolic chambers, in closed (56 species) or flow-through (two species) systems (electronic supplementary material, table S1). If a study reported more than one metabolic value (for a given temperature) for the same species and collection site, we estimated (and used in the analyses) the mean value. Given that most of the studies in our database were conducted during the '70s, in some works the collection sites were reported in a relatively

ambiguous way (e.g. only the state or the province of collection is provided). For that reason, we decided to use geographic latitude as the only explanatory variable, instead of to perform more detailed analyses based on, for example, climatic variables (see Discussion).

We used the equation $Q_{10} = (Q_2/Q_1)^{10/(T_2-T_1)}$ to estimate $Q_{10\text{-SMR}}$ values, where Q_2 (or T_2) and Q_1 (or T_1) are the metabolic rates (or ambient temperatures) at the highest and lowest ambient temperature, respectively [16]. We estimated $Q_{10\text{-SMR}}$
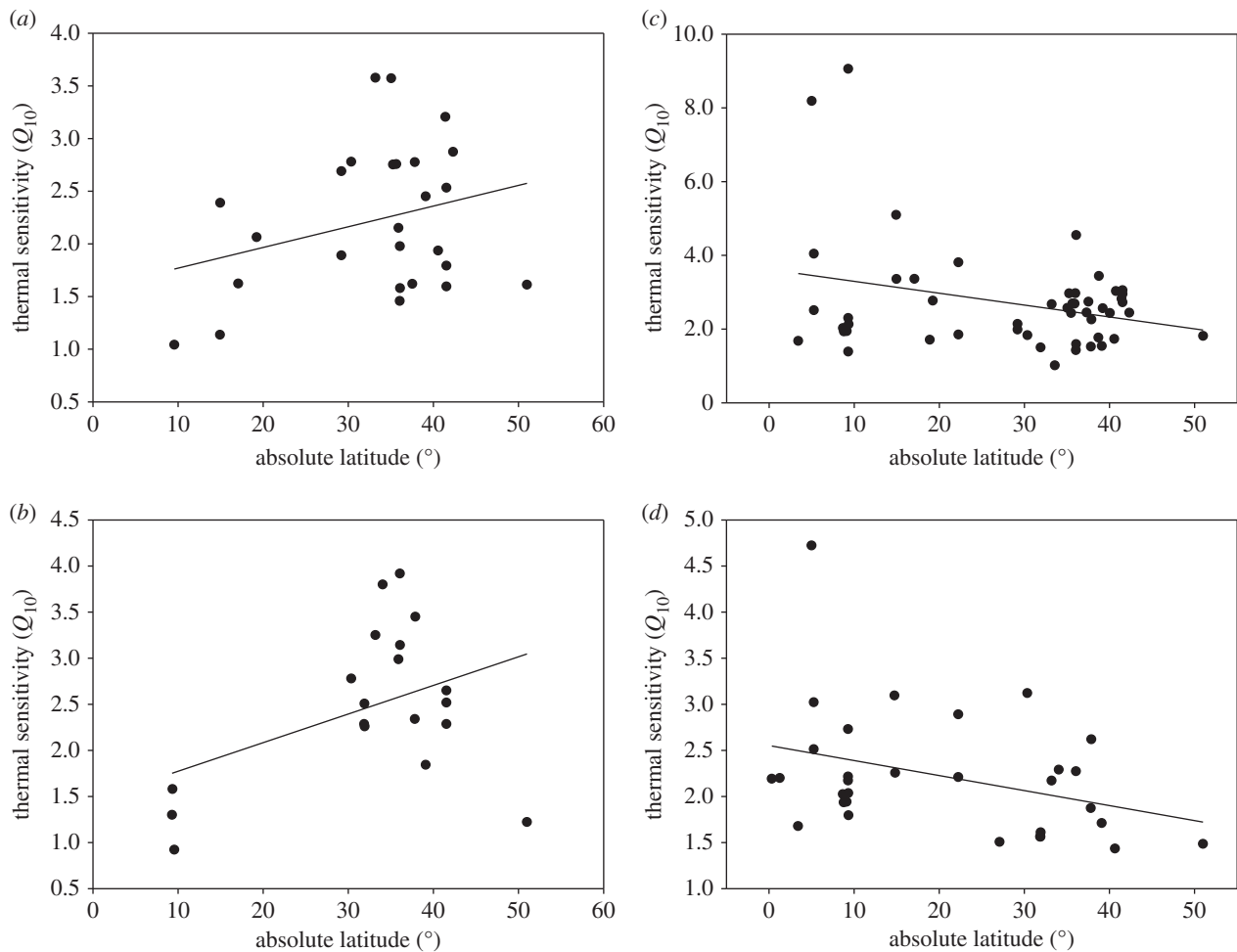
Figure 3. Relationship between Q_{10} values and absolute latitude for different thermal ranges: (a) $5-15°C$, (b) $10-20°C$, (c) $15-25°C$ and (d) $20-30°C$.

Table 1. Sample size (N), slope (±1 s.e.), coefficient of determination (r^2), phylogenetic signal (λ), and p-value (p), obtained with conventional and phylogenetic methods, for the relationship between $Q_{10\text{-SMR}}$ (estimated at four thermal ranges) and absolute latitude.

		conventional analysis			phylogenetic analysis		
thermal range	N	slope (s.e.)	r^2	p	slope (s.e.)	λ	p
$5-15°C$	26	0.020 (0.014)	0.08	0.16	0.020 (0.014)	0	0.17
$10-20°C$	19	0.031 (0.016)	0.17	0.08	0.031 (0.016)	0	0.06
$15-25°C$	50	−0.032 (0.015)	0.08	0.04	−0.028 (0.020)	0.47	0.16
$20-30°C$	31	−0.016 (0.008)	0.12	0.05	−0.016 (0.008)	0	0.05

values at four different thermal ranges: $5-15°C$ (Q_{5-15}: 26 data from 25 species), $10-20°C$ (Q_{10-20}: 19 data from 18 species), $15-25°C$ (Q_{15-25}: 50 data from 48 species), and $20-30°C$ (Q_{20-30}: 31 data from 30 species). In 92 cases, $Q_{10\text{-SMR}}$ values were estimated from a 10°C difference between experimental temperatures, while in 34 cases $Q_{10\text{-SMR}}$ values were estimated from a less than 10°C difference (typically 5°C) between experimental temperatures (electronic supplementary material, table S1). In this sense, note that the equation used to estimate Q_{10} values accounts for differences in the thermal range over which they are calculated.

(b) Statistical analyses

Conventional as well as phylogenetic analyses were used to evaluate: (i) the relationship between $Q_{10\text{-SMR}}$ and absolute latitude (hereafter AbsLat), (ii) the relationship between the experimental temperature at which the highest $Q_{10\text{-SMR}}$ value ($T_HQ_{10\text{-SMR}}$) for each species is observed and AbsLat, and (iii) if $Q_{10\text{-SMR}}$ values of temperate species (AbsLat > 22.5°) differed from those of tropical species (AbsLat < 22.5°). In conventional analyses, we calculated the slope (and associated statistics) of the relationship between $Q_{10\text{-SMR}}$ and AbsLat separately for each thermal range, using generalized least-squares regressions. For those species with two or more $Q_{10\text{-SMR}}$ values, we recorded the experimental temperature (the middle-point of the thermal range) at which the highest value of $Q_{10\text{-SMR}}$ was observed ($T_HQ_{10\text{-SMR}}$). Then, we calculated the slope (and associated statistics) between this experimental temperature and AbsLat, through a generalized least-squares regression. Finally, we used one-way ANOVAs to evaluate differences in $Q_{10\text{-SMR}}$ between temperate and tropical species; again, this was done separately for each thermal range. For phylogenetic analyses, we first trimmed four phylogenetic trees (one for each thermal range; figure 2 and supplementary material, Kreiman_PhylogeneticTree.tre), based on the tree published in [28]. There were five species present in our dataset that
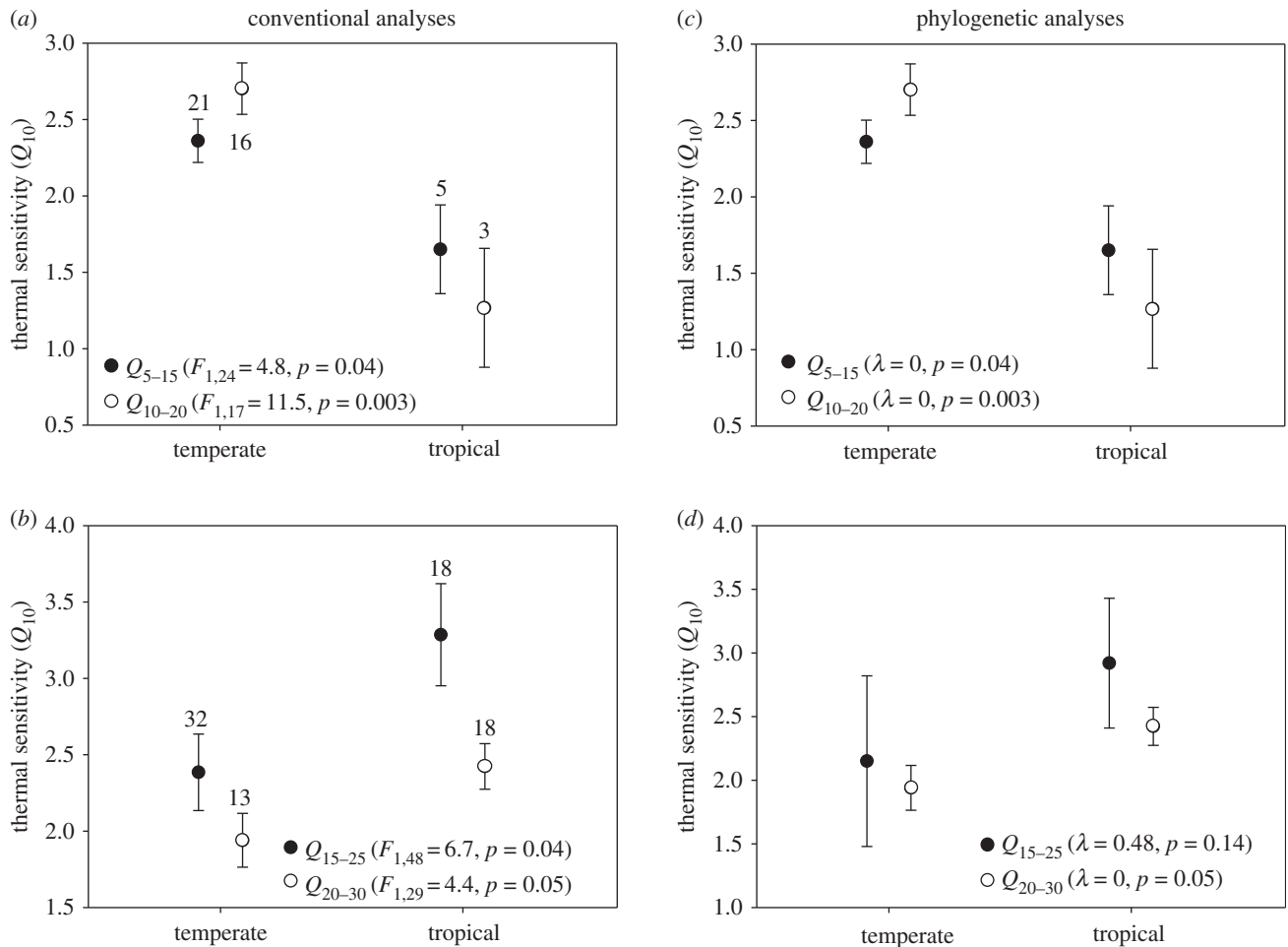
Figure 4. Comparisons of thermal sensitivity values (Q_{10}) between temperate and tropical species, estimated at different thermal ranges: (*a*) and (*c*) $5-15°C$ and $10-20°C$, (*b*) and (*d*) $15-25°C$ and $20-30°C$. Sample size for each group is given above or below the error bars (which represent $\pm$ 1 s.e.) in the left panels.

were not available in [28]; in these cases, we used the closest related species that was present in the tree: *Gyrinophilus palleucus* instead of *G. danielsi*, *Ichthyophis orthoplicatus*, instead of *I. kohtaoensis*, *Ingerana baluensis* instead of *Ooeidozyga laevis*, *Physalaemus gracilis* instead of *P. fuscomaculatus*, and *Thorius troglodytes* instead of *Thorius* sp. Then, using phylogenetic generalized least-squares regressions (PGLS; [29]), we evaluated the association between $Q_{10\text{-SMR}}$ and AbsLat as well as differences between temperate and tropical species in $Q_{10\text{-SMR}}$ values, separately for each thermal range. Also, we used a PGLS regression to evaluate the association between $T_H Q_{10\text{-SMR}}$ and AbsLat (supplementary material, Kreiman_Script.R). We estimated the influence of phylogenetic relationships on $Q_{10\text{-SMR}}$ by calculating Pagel's λ, optimized using maximum-likelihood transformation. Pagel's λ is a measure of the phylogenetic signal of the residuals of a regression model [30], which can range from 0 (no correlation between species) to 1 (correlation between species equal to the Brownian expectation) [31–33]. Conventional analyses were conducted with the package 'stats', while phylogenetic analyses were conducted with the packages 'caper' [33] and 'APE' [34] in the free software R [35]. Statistical significance was established at the 0.05 level, and probability values between 0.05 and 0.1 were considered as marginally significant (i.e. indicative of a trend).

3. Results

At lower ambient temperatures, we found a marginally significant positive correlation between Q_{10-20} values and absolute latitude, but not between Q_{5-15} values and AbsLat (table 1, figure 3*a,b*). In contrast, at higher ambient temperatures, there was a negative and significant correlation

between $Q_{10\text{-SMR}}$ values and AbsLat for both thermal ranges (table 1; figure 3*c,d*). In line with this, both Q_{5-15} and Q_{10-20} were significantly higher in temperate than in tropical species (figure 4*a*), while Q_{15-25} and Q_{20-30} were higher in tropical than in temperate species (figure 4*b*). In addition, there was a significant negative correlation between $T_H Q_{10\text{-SMR}}$ values and AbsLat (slope (s.e.) = -0.192 (0.059), $r^2 = 0.22$, $p = 0.003$).

Regarding the phylogenetic analyses, we found that, although all the relationships between $Q_{10\text{-SMR}}$ values and latitude have the predicted direction (i.e. positive at low T_As and negative at high T_As), only one was statistically significant (Q_{20-30}), while another reaches a marginal probability value (Q_{10-20}) (table 1). As was found with the conventional analyses, the Q_{5-15} and Q_{10-20} values of temperate species were significantly higher than those of tropical species (figure 4*c*). Conversely, the Q_{15-25} and Q_{20-30} values of tropical species were higher than those of temperate species, yet these differences only were significant for the thermal range of $20-30°C$ (figure 4*d*). Finally, the negative correlation between $T_H Q_{10\text{-SMR}}$ values and AbsLat was also significant in the phylogenetic analysis (slope (s.e.) = -0.192 (0.060), $r^2 = 0.22$, $\lambda = 0$, $p = 0.003$).

4. Discussion

For more than a century, the different hypotheses regarding physiological compensatory mechanisms that permit terrestrial ectotherms to face the decrease in T_A that occurs

toward higher latitudes have been assessed [7,8]. In the case of amphibians, for example, several studies showed that, in line with the 'metabolic cold adaptation' hypothesis, boreal and temperate species exhibit higher SMRs than tropical species, for a wide range of experimental T_As [36–41]. In addition, a similar correlation between SMR and T_A has been documented for populations of a single species assessed at different latitudes [42,43] and for different species occurring at different altitudes [44]. Moreover, in line with the 'climatic variability' hypothesis, latitudinal patterns of acclimation in the SMR of amphibians also agree with the natural pattern of variation in T_A. That is, acclimatization responses in oxygen consumption to changes in T_A are more common in temperate species—which usually experience large thermal fluctuations in their habitats—than in tropical species—which usually experience small thermal fluctuations in their habitats [43–46]. Overall, these results suggest that SMR of amphibians has been shaped by climatic factors, through natural selection (but see [47,48]).

The role of spatial patterns in other compensatory mechanisms, like adjustments in thermal sensitivity of metabolic rates, nevertheless has received much less attention. In fact, an early debate that took place between authors who claimed that $Q_{10\text{-SMR}}$ was independent of latitude [36,37,49] and authors suggesting that tropical ectotherms tend to have higher $Q_{10\text{-SMR}}$ than temperate species [50–52] was abandoned unsolved. Thus, in the present study, we have advanced on previous knowledge on this topic by showing that the relationship between $Q_{10\text{-SMR}}$ and latitude changes with the thermal range over which $Q_{10\text{-SMR}}$ is assessed, as expected from the 'metabolic homeostasis' hypothesis (HMM). Specifically, we found that temperate species have higher $Q_{10\text{-SMR}}$ than tropical species for the colder end of the thermal range (i.e. 5–15°C and 10–20°C), while the opposite is true for the warmer end of the thermal range (i.e. 15–25°C and 20–30°C). Moreover, we found a negative correlation between the experimental temperature at which $Q_{10\text{-SMR}}$ of each species is maximal and geographical latitude. That is, the highest $Q_{10\text{-SMR}}$ values occur at lower experimental temperatures as ambient temperature decreases. We believe that, as proposed by the HMM, this pattern of variation is related to the benefits of reducing maintenance costs when T_A is too low to allow activity [53,54], and to rapidly exploit short-term temporal windows of favourable T_As during the warmer hours of the day [13,55,56]. Interestingly, similar changes in the $Q_{10\text{-SMR}}$ values, but on a seasonal basis, have been reported as an adaptation of hibernating anurans to seasonal environments, as these changes may allow animals to recover faster after the long period of winter quiescence [57,58]. Moreover, a reduction in Q_{10} values during dormancy has been suggested as an adaptive response to conserve energy stores during aestivation in anurans [59]. All this evidence reinforce the idea that the thermal reaction norms of the standard metabolic rate might be the actual targets of natural selection and not a particular value for a given temperature ([2], but see also [48]).

Another interesting result emerging from our analyses is that variation in $Q_{10\text{-SMR}}$ values, as a function of the experimental thermal range, was greater for tropical species (from 1.27 to 3.29) than for temperate species (from 1.94 to 2.70). In effect, using the formula relating Q_{10} values to activation energy (E_t) [60], it is observed that, although the mean value of E_t is similar for tropical (mean $\pm$ s.e. = 0.58 $\pm$
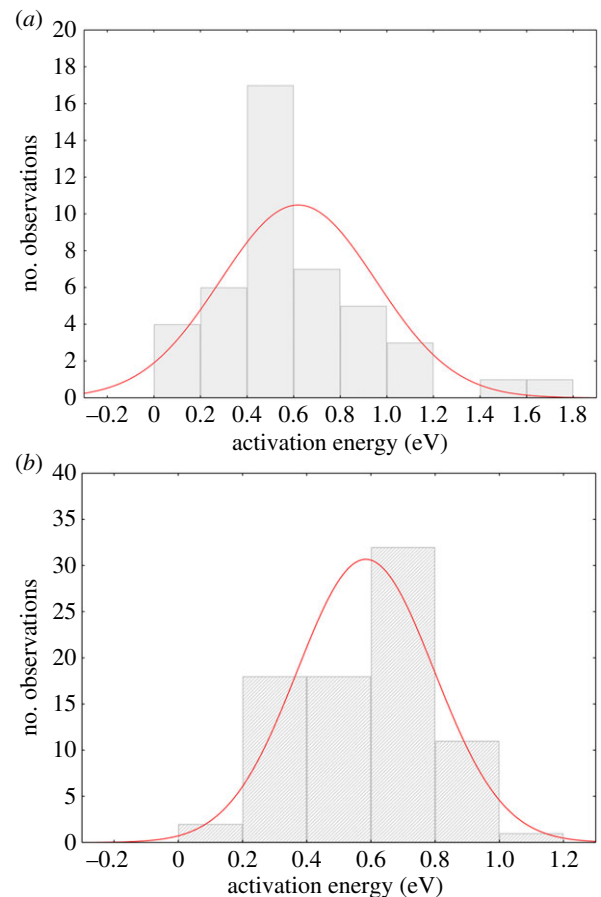

Figure 5. Distribution of activation energies (E_t) for (*a*) tropical and (*b*) temperate species. (Online version in colour.)

0.30 eV, $n = 44$) and temperate species (mean $\pm$ s.e. = 0.62 $\pm$ 0.39 eV, $n = 82$), variation in E_t is greater for the former group (Levene's test: $F_{1,124} = 4.3$, $p = 0.04$). In any case, leaving aside this difference in activation energy between geographical zones, we recall that almost all estimations of E_t, for both tropical (figure 5*a*) and temperate species (figure 5*b*), encompass the range of activation energies expected for biochemical reactions (from *ca* 0.2 to 1.2 eV) [60,61].

Finally, it is important to note three potential caveats about our study. First, given that several works included in the dataset only reported $Q_{10\text{-SMR}}$ for one thermal range, we were unable to analyse our data based on the concept of thermal performance curves (i.e. to fit a curve to the data). Second, tropical species are often not able to survive at temperatures where temperate and boreal species usually occur [37], restricting the number of data points for tropical species measured at the colder end of the thermal range. This imbalance in the number of species measured at lower temperatures could cause some bias in the comparison of $Q_{10\text{-SMR}}$ between geographical zones. Third, given that some studies reported collection sites in a fairly ambiguous way, we decided to use latitude as the single explanatory variable, instead of performing more detailed analyses based on, for example, climatic variables. However, we note that there are several reasons that could favour the use of latitude, over environmental data, in the search for large-scale patterns of variation in phenotypic traits. For instance, it has been suggested that latitude is probably a better predictor of long-term regimens of climatic variables than current climate values provided by weather stations [62]. In addition, latitude

is correlated with several other environmental factors that are often not assessed, such as climatic (e.g. wind speed), ecological (e.g. day length, environmental productivity) and historical factors (e.g. geographical boundaries), that could affect physiological traits [63]. Finally, given that species included in physiological studies usually occur over geographical areas that are much larger than collection sites, adaptation to environmental conditions should match the conditions taking place over these large areas (if genetic flow among sites occurs). Consequently, latitude may represent a weighted variable of the overall climatic conditions acting over spatial scales that are much closer to the scale at which physiological adaptation is expected to occur than the conditions observed at any particular collection site [8,64]. All these three, not mutually exclusive, reasons could be behind the fact that geographical latitude usually fits to the data as well as climatic variables, despite the fact that climatic variables (and not latitude) are the ultimate factors underlying physiological variation [8,65].

To sum up, the present work shows that the relationship between $Q_{10\text{-SMR}}$ and latitude in ectothermic animals changes with the thermal range over which $Q_{10\text{-SMR}}$ is assessed, and that these changes are in agreement with an adaptive hypothesis (the 'metabolic homeostasis' hypothesis). Despite this, we recognize that the pattern identified here requires more data and further analyses in order to be confirmed.

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