When torpid animals arouse and warm up to restore normal body temperature ($T_b$), they produce heat at levels that can reach up to 10 times basal metabolic rate (BMR), close to the cold-induced summit metabolism (VO$_2$-sum). Because torpor is an adaptation aimed at conserving energy over periods of low ambient temperature ($T_a$) and food availability, selective forces that have led to the evolution of torpor may have simultaneously favoured high thermogenic capacity (i.e. VO$_2$-sum) relative to the maintenance costs (i.e. BMR), hence a higher factorial aerobic scope (FAS; the ratio of VO$_2$-sum to BMR). My objective was to test this adaptive hypothesis using a phylogenetically informed comparative approach with data on BMR and VO$_2$-sum in rodents. I found a strong negative correlation between FAS and the average of the daily minimum $T_a$ ($T_{min}$) in species using torpor, which was due to differential effects of $T_a$ on BMR (but not VO$_2$-sum) in species that use torpor compared with species that do not. In addition, FAS was negatively correlated with the lowest torpid $T_b$ in a subset of nine species. These results suggest that in species using torpor, selective forces may have acted to maximize the efficiency of thermogenic capacity (VO$_2$-sum) relative to maintenance costs (BMR), resulting in an increasing FAS with decreasing $T_a$.

1. Introduction

Animals have adapted in many ways to survive periods of cold ambient temperature ($T_a$) and low food availability, such as food hoarding, migration and hibernation. For many species, torpor (a state of inactivity and reduced responsiveness usually characterized by a reduced body temperature ($T_b$) and rate of metabolism) is a key physiological adaptation to life in cold environments with insufficient food availability [1]. While torpor allows the animal to conserve energy over protracted periods, it generally requires high levels of metabolic rate during arousals to warm up from torpid to normothermic $T_b$ [2]. In eastern chipmunk (Tamias striatus) for example, energy expenditure during arousals can reach up to 10 times the euthermic basal metabolic rate (BMR [3,4]), close to the cold-induced summit metabolism (VO$_2$-sum) reported in this species [5]. Therefore, selective forces that led to the evolution of torpor may have simultaneously favoured high thermogenic capacity (to warm up quickly from torpor) relative to maintenance costs during torpor (to conserve energy), which itself may be linked to a low BMR during non-torpor periods. Hence, species using torpor should have a higher factorial aerobic scope (FAS; ratio of VO$_2$-sum to BMR) than homeothermic species. There have been many comparative studies on the selective forces that led to the evolution of metabolic rates that deviate substantially from allometric predictions [6,7]. Latitude and $T_b$ have been repeatedly shown to be correlated with metabolism, with species living at extreme latitudes and in cold environments
having higher daily energy expenditure, BMR and VO₂-sum [7–9]. Moreover, species using torpor have lower BMR during non-torpor periods than euthermic species, as suggested by two large (but non-phylogenetic) comparative analyses in 533 bird and 639 mammal species, respectively [10,11]. The relationship between torpor use and VO₂-sum, however, has never been verified. Much less attention has been paid to factors causing interspecific differences in metabolic flexibility. In the only study that did so, the absolute aerobic scope (AAS; mass-specific VO₂-sum minus mass-specific BMR) was significantly correlated with Tₐ and latitude, but not with torpor use [12]. This is surprising, because metabolic flexibility (i.e. FAS or AAS) should be a particularly important physiological feature of species using torpor (see above).

An overlooked aspect of previous comparative analyses of BMR and VO₂-sum is the potential interaction between torpor use and Tₐ and/or latitude. While BMR and VO₂-sum generally increase at low Tₐ or high latitudes [7], it may not necessarily be the case for species using torpor. One may expect that a low metabolic rate during torpor will maximize chances of survival on limited energy stores (body fat or larder hoards). In turn, a low torpid metabolic rate may be linked to low maintenance cost during non-torpor periods (i.e. BMR). By contrast, one may expect that the effect of Tₐ and/or latitude on VO₂-sum is more pronounced in heterothermic than homeothermic species, because torpid hibernators may want to re-warm from their low Tₐ in the least possible time, which is limited by the metabolic machinery underlying VO₂-sum [2]. Once combined, the differential effect of Tₐ and/or latitude on BMR and VO₂-sum should yield large differences in FAS, but only in species using torpor. Here, I used a phylogenetically informed comparative approach [13,14] to test whether rodent species using torpor in cold climates have evolved different metabolic machineries by looking at how torpor use and Tₐ interact to determine BMR, VO₂-sum and FAS.

2. Material and methods

(a) Metabolic and environmental data

I used a recently compiled dataset [7] on VO₂-sum elicited by cold exposure in a mixture of helium and oxygen (i.e. heliox), a widely used method to measure the thermogenic capacity of wild endotherms [15]. In addition to VO₂-sum, this dataset includes a measure of BMR or resting metabolic rate (RMR) in the same population and, when possible, in the same individuals captured in the field or maintained for only a few generations in the laboratory. In this dataset, RMR measures are not significantly higher than BMR measures (see below and [7]); hence the term BMR is used hereafter. Most animals were acclimatized to the least possible time, which is limited by the metabolic machinery underlying VO₂-sum [2]. Once combined, the differential effect of Tₐ and/or latitude on BMR and VO₂-sum should yield large differences in FAS, but only in species using torpor. Here, I used a phylogenetically informed comparative approach [13,14] to test whether rodent species using torpor in cold climates have evolved different metabolic machineries by looking at how torpor use and Tₐ interact to determine BMR, VO₂-sum and FAS.

several environmental variables were inter-correlated, especially Tₐ min, Tₐ max latitude and altitude. Here I focused on Tₐ max because it is the most relevant variable in light of torpor use and was the best predictor of VO₂-sum (multiple r² with body mass = 0.776) and second best of BMR (the correlation between Tₐ min and Tₐ max is 0.84) [7], but I replicated all analyses using Tₐ max and altitude, precipitation, latitude and corrected latitude [12]. I calculated FAS as the ratio of VO₂-sum to BMR and included a new variable coding for torpor use (yes or no) for each species (see the electronic supplementary material, table S1). To this end, I followed categorization by McNab [10] except for eight species that were not in common (see the electronic supplementary material). I found data on both normothermic and minimum torpid Tₐ on nine of the 12 species considered here as using torpor (see the electronic supplementary material).

(b) Statistical analysis

I analysed log-transformed BMR, log-transformed VO₂-sum and FAS as function of log-transformed body mass (Mₐ), Tₐ min, torpor use and the interaction between Tₐ min and torpor use. To take phylogenetic relationships into account, I analysed the data using phylogenetic generalized least squares (PGLS) in REGRESSIONv2.M [14] and a phylogenetic supertree which contained all species in the dataset (see the electronic supplementary material, figure S1). I ran all models using three different transforms: Pagel’s λ, Grafen’s ρ and Ornstein–Uhlenbeck (see [14] for derived terminology), but retained models using Pagel’s λ transform because they fit the data better according to the Akaike information criterion. The final sample size used in each analysis was 58 (i.e. 41 homeothermic and 17 heterothermic species/populations), but for all models, I subtracted one d.f. for every soft polytomy contained in the phylogeny [16]. A variable coding for whether all the requirements were fulfilled for BMR or not (RMR) was not significant for either BMR or Tₐ max because torpor use was significant, indicating that species using torpor have lower BMR at a Tₐ min of 0°C (i.e. intercept; table 1a). The interaction between Tₐ min and torpor use was positive but not significant (table 1a). The correlation (r) between mass-residual BMR and Tₐ min was −0.53 in species that do not use torpor, but only −0.05 in homeothermic species (figure 1a).

Cold-induced VO₂-sum was positively correlated with Mₐ (table 1b). In contrast to BMR, VO₂-sum was negatively correlated with Tₐ min and the main effect of torpor use was not significant (table 1b). The interaction between Tₐ min and torpor use on VO₂-sum was negative but not significant (table 1b). The correlation (r) between mass-residual VO₂-sum and Tₐ min was −0.59 in species that do use torpor and −0.55 in homeothermic species (figure 1b).

FAS was not significantly correlated with Mₐ but negatively correlated with Tₐ min (table 1c). The main effect of torpor use was significant, indicating that species using torpor have higher FAS at a Tₐ min of 0°C (i.e. intercept; table 1c). There was a significant interaction between torpor use and Tₐ min on FAS (table 1c). While FAS is independent of Tₐ min in homeothermic species (r = −0.09), it increases steeply with decreasing Tₐ min in species that use torpor (r = −0.76; figure 1c). Similar results are obtained with Tₐ max (i.e. significant interaction with torpor use; electronic

3. Results

BMR was positively correlated with Mₐ but not with Tₐ min (table 1a). The main effect of torpor use was significant, indicating that species which use torpor have lower BMR at a Tₐ min of 0°C (i.e. intercept; table 1a). The interaction between Tₐ min and torpor use was positive but not significant (table 1a). The correlation (r) between mass-residual BMR and Tₐ min was −0.53 in species that do not use torpor, but only −0.05 in homeothermic species (figure 1a).

Cold-induced VO₂-sum was positively correlated with Mₐ (table 1b). In contrast to BMR, VO₂-sum was negatively correlated with Tₐ min and the main effect of torpor use was not significant (table 1b). The interaction between Tₐ min and torpor use on VO₂-sum was negative but not significant (table 1b). The correlation (r) between mass-residual VO₂-sum and Tₐ min was −0.59 in species that do use torpor and −0.55 in homeothermic species (figure 1b).

FAS was not significantly correlated with Mₐ but negatively correlated with Tₐ min (table 1c). The main effect of torpor use was significant, indicating that species using torpor have higher FAS at a Tₐ min of 0°C (i.e. intercept; table 1c). There was a significant interaction between torpor use and Tₐ min on FAS (table 1c). While FAS is independent of Tₐ min in homeothermic species (r = −0.09), it increases steeply with decreasing Tₐ min in species that use torpor (r = −0.76; figure 1c). Similar results are obtained with Tₐ max (i.e. significant interaction with torpor use; electronic
However, neither altitude, latitude nor precipitation significantly interacted with torpor use to influence FAS, although the interaction with latitude corrected for altitude was marginally non-significant (see the electronic supplementary material, table S2).

Although FAS was not correlated with normothermic $T_b$ ($t_{14} = 0.74; p = 0.47$), it was significantly negatively correlated with torpid $T_b$ (PGLS estimate $s.e. = -0.20 \pm 0.05; t_{14} = 2.25; p = 0.041$; figure 2).

### 4. Discussion

Given that torpor is a physiological adaptation aimed at conserving energy, but involves high levels of thermogenesis during it, it could be hypothesized that the use of torpor may have a negative impact on FAS, as it requires additional energy expenditure. However, the results from the phylogenetic least squares analysis suggest that this is not the case, as neither altitude, latitude, nor precipitation significantly interacted with torpor use in influencing FAS. Although FAS was not correlated with normothermic $T_b$, it was significantly negatively correlated with torpid $T_b$, indicating that the use of torpor during cold conditions can negatively impact FAS. This finding highlights the importance of considering the role of torpor in understanding metabolic responses to environmental factors, as it may provide an additional layer of adaptation to cold environments.
arousals [2], it is intuitive to think that it requires high thermogenic capacity (VO_{2-sum}) relative to maintenance costs (BMR), leading to a high FAS. Analysing FAS resulted in otherwise undetectable from the individual analyses of its components (i.e. BMR and VO_{2-sum}). In homeothermic species, both BMR and VO_{2-sum} were negatively correlated with T_{min}, hence there was no correlation between T_{min} and FAS. In heterothermic species using torpor, BMR was independent of T_{min}, while the correlation was still present with VO_{2-sum}. Once combined, these effects yielded a strong negative relationship between T_{min} and FAS in heterothermic species ($r^2 = 0.57$; figure 1c).

The different effect of T_{min} on BMR and FAS in heterothermic versus homeothermic species may reflect the presence of selective forces that acted on the metabolic machinery of heterothermic species using torpor in cold climates.

The comparative method cannot unambiguously demonstrate ‘adaptation,’ meaning the tailoring of an ancestral condition to allow it to meet the needs of a new physical environment [13]. It is possible that high FAS in species using torpor in cold climates simply represents phenotypic plasticity, whereby individuals adjust their FAS in response to local environments. Further studies are needed to differentiate genetic and population-level adaptations from phenotypic plasticity. An interesting avenue for future research would be to test if factors that negatively affect FAS also reduce survival in species using torpor in cold climates. Parasites for example, are known to increase RMR, decrease VO_{2-sum} and consequently FAS in free-ranging eastern chipmunks [5]. Perhaps not coincidentally, parasites also reduce overwinter survival in juvenile chipmunks during a year of low food abundance [17]. Moreover, in a captive chipmunk population, BMR during periods of nonthermogenic arousal (i.e. between torpor bouts) was negatively correlated with total number of torpor bouts exhibited throughout the winter [4]. Given that a high FAS was associated with a low torpid T_b across rodent species (figure 2), the current empirical evidence suggests that torpor is an energy conservation mechanism that simultaneously requires low maintenance costs and high thermogenic capacity. Another interesting avenue for future research will be to test whether BMR during non-torpor periods is linked to metabolism while torpid.

I was supported by a postdoctoral scholarship from NSERC. I thank M. Humphries and M. Chappell for comments on a previous draft of this manuscript.

References


