

A comparative analysis of thermogenic capacity and cold tolerance in small birds

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Summary

Small birds showing marked seasonal changes in cold tolerance also exhibit winter increases in summit metabolic rate (M_{sum} =maximum cold-induced thermogenesis or thermogenic capacity) relative to summer birds. However, some birds show modest seasonal changes in cold tolerance without winter increases in M_{sum} and others exhibit large seasonal changes in cold tolerance with only minor changes in M_{sum} . Thus, the degree of correlation between cold tolerance and M_{sum} is uncertain and no interspecific study has directly addressed this question. In this study, we measured cold tolerance and M_{sum} in summer- (21 species) and winter- (11 species) acclimatized birds from southeastern South Dakota. M_{sum} was measured as the maximum oxygen consumption attained during exposure of individual birds to a declining series of temperatures in 79% helium/21% oxygen (helox). Cold tolerance was measured as the temperature at cold

limit (T_{CL}), which is the helox temperature that induced hypothermia in individual birds. Residuals from allometric regressions of $\log M_{\text{sum}}$ and $\log T_{\text{CL}}$ were significantly and negatively related for summer ($R^2=0.34$, $P=0.006$) and winter ($R^2=0.40$, $P=0.037$) birds. Data were also subjected to a comparative analyses with phylogenetically independent contrasts to remove potential confounding effects of phylogeny, and results were similar to the non-phylogenetic analyses, with significant negative correlations in both summer ($R^2=0.47$, $P<0.001$) and winter ($R^2=0.40$, $P=0.049$). Thus, birds with high M_{sum} tended to show reduced T_{CL} (i.e. high cold tolerance), suggesting that cold tolerance and summit metabolism are phenotypically linked in small birds.

Key words: thermogenesis, cold, bird, acclimatization, comparative analysis.

Introduction

Small birds wintering in temperate regions generally show markedly improved cold tolerance capabilities relative to summer-acclimatized birds (Marsh and Dawson, 1989; Swanson, in press). This winter acclimatization primarily results from an increased ability to sustain high levels of shivering thermogenesis over prolonged periods (Marsh and Dawson, 1989). In birds showing marked winter improvement of cold tolerance, this improvement is also associated with expanded M_{sum} (summit metabolism or thermogenic capacity), typically measured by indirect calorimetry as the maximal rate of oxygen consumption under cold stress (Dawson and Smith, 1986; Marsh and Dawson, 1989; Swanson, 1990a; Cooper and Swanson, 1994; Liknes and Swanson, 1996; Liknes et al., 2002; Cooper, 2002). Furthermore, birds that show relatively minor seasonal differences in cold resistance also show no, or only minor, seasonal differences in M_{sum} (Dawson et al., 1983a; Saarela et al., 1989, 1995; Swanson and Weinacht, 1997). Thus, during winter acclimatization the expanded M_{sum} is closely associated with increased shivering endurance at submaximal levels of cold challenge. Indeed, Swanson (2001) demonstrated that shivering endurance under a standardized

cold exposure was positively correlated with M_{sum} in three species of small passerines. Moreover, expanded endurance is tied to enhanced maximal capacities for aerobic activity in vertebrate animals generally (Bennett, 1991).

Nevertheless, seasonal changes in shivering endurance and cold resistance in some species of small birds may occur without corresponding changes in M_{sum} , and geographic variation in cold resistance is not always associated with variation in M_{sum} (Dawson et al., 1983a; Swanson, 1993). Thus, cold tolerance and M_{sum} do not always change in lockstep and the extent of their phenotypic correlation is uncertain. Shivering endurance and M_{sum} are correlated intraspecifically in small birds (Swanson, 2001), but the interspecific relationship between cold tolerance and M_{sum} has not been directly examined for birds. Intraspecific seasonal changes in cold tolerance in birds are concluded either when birds tolerate a static cold exposure longer in winter than in summer (e.g. Dawson and Carey, 1976; Dawson and Smith, 1986; Cooper and Swanson, 1994) or when colder temperatures are required to induce hypothermia in winter than in summer (Saarela et al., 1989, 1995; Liknes et al., 2002). Efforts to test the interspecific relationship between cold

tolerance and M_{sum} have not yet been undertaken, and are potentially confounded by body size effects on metabolic rates and heat loss. Testing the relationship between M_{sum} and cold tolerance requires a standardized cold exposure among species and measurement of either shivering endurance or the temperature inducing hypothermia. Developing a standardized measure of shivering endurance requires a standardized cold challenge for all species measured, which is difficult, if not impossible, to attain because factors such as body size and thermal conductance vary among species and greatly impact heat loss to the environment (Aschoff, 1981). One way around this problem, however, is to hold shivering endurance essentially constant while measuring the temperature in helox (79% helium/21% oxygen) required to elicit hypothermia (or the temperature at the cold limit, T_{CL} ; after Saarela et al., 1989).

The objective of this study was to examine the interspecific relationship between cold tolerance (measured as T_{CL} under a sliding helox cold exposure) and M_{sum} in both summer and winter in a phylogenetically diverse sample of small birds. We used both standard and phylogenetically corrected methods to analyze the interspecific $M_{\text{sum}}/T_{\text{CL}}$ relationship to determine whether phylogeny influenced any correlation between M_{sum} and T_{CL} . To our knowledge, this is the first study to directly examine, using relevant comparative techniques, whether an interspecific phenotypic correlation between cold tolerance and M_{sum} exists for birds.

Materials and methods

Birds and collection

We measured M_{sum} and T_{CL} for 21 species in summer and 11 species in winter (Table 1); these species ranged in body mass M_b from 9.2 to 62.6 g. All birds used in the present study were captured by mist net in summer (late April–August) or winter (December–February) near Vermillion, Clay County, South Dakota, USA (42°47'N, 97°0'W). The species used in this study are all common summer, winter or permanent residents in this area. Following capture, birds were transported to the laboratory where they were held at room temperature (22°C), with food (bird seed, mealworm larvae and/or mulberries) and water *ad libitum*, until cold exposure tests later on the day of capture. Body mass was measured to the nearest 0.1 g immediately prior to cold exposure tests, which were conducted from 09:00 h–20:00 h CST in summer and from 10:00 h–19:00 h CST in winter. Following cold exposure tests, birds were banded with a standard US Fish and Wildlife Service leg band and released at the site of capture. M_{sum} and T_{CL} were measured only once on individual birds.

Measurement of cold tolerance and M_{sum}

Standardized conditions for determining T_{CL} must be delineated to use cold tolerance data for comparative analyses. Swanson et al. (1996) suggested standard methods for eliciting M_{sum} in birds by sliding cold exposure in helox that we adapted for measurement of T_{CL} . Using this method, we exposed an individual bird to a declining series of temperatures in 79%

helium/21% oxygen (helox), where temperature was decreased by 3°C at 25 min after the initiation of cold exposure, and every 20 min thereafter, until hypothermia was induced. We concluded that hypothermia had occurred when oxygen consumption decreased steadily, without rebounding, over several minutes, reaching levels lower than those recorded over the preceding portion of the cold exposure test. To verify hypothermia, we removed birds from the metabolic chamber and measured body temperature with a Cole-Parmer Model 8500-40 Thermocouple Thermometer (Chicago, IL, USA) by inserting a lubricated 20-gauge copper–constantan thermocouple into the cloaca to a depth (approximately 1 cm) where further insertion did not alter the temperature reading. We considered birds with body temperature $T_b < 37^\circ\text{C}$ as hypothermic, and birds were invariably hypothermic when the conditions noted above had been met. We defined the helox temperature at the beginning of this steady decline in oxygen consumption as T_{CL} . One further matter in the standardization of T_{CL} measurement involves the temperature at which the sliding helox cold exposure is initiated. Because T_{CL} is affected by body mass, to keep thermogenic endurance roughly standardized among species, cold exposure tests must begin at higher temperatures for smaller birds.

Based upon previous studies using both sliding and static helox cold exposure in both summer- and winter-acclimatized birds (Dawson and Smith, 1986; Swanson, 1990a, 1993; Cooper and Swanson, 1994; O'Connor, 1995a; Dutenhoffer and Swanson, 1996; Liknes and Swanson, 1996; Swanson et al., 1996), we initially measured T_{CL} by sliding helox cold exposure for nine species of summer-acclimatized passerines (*Contopus virens*, *Tyrannus tyrannus*, *Vireo gilvus*, *Dumetella carolinensis*, *Troglodytes aedon*, *Carduelis tristis*, *Spizella pusilla*, *Dendroica petechia* and *Pheucticus ludovicianus*) ranging from 9.6 to 40.7 g mean body mass, and for five species of winter-acclimated birds (*Picoides pubescens*, *P. villosus*, *Sitta carolinensis*, *Cardinalis cardinalis* and *Passer domesticus*) ranging from 21.8 to 62.6 g mean body mass. For T_{CL} measurements on these species, sliding helox cold exposure was initiated either (1) at 6–8°C above temperatures producing hypothermia in a majority of individuals within 1 h in previous studies using static cold exposure, (2) at 6–8°C above T_{CL} from previous studies using sliding cold exposure, or (3) if cold tolerance had not previously been measured for that species, from extrapolations based on body mass from previous studies on other species. From these T_{CL} data, we calculated mean T_{CL} for each of these species and generated an allometric equation predicting T_{CL} for both summer- and winter-acclimatized birds:

$$\text{Summer: } T_{\text{CL}} = 295.1M_b^{-0.026} - 273 \quad (N=9, R^2=0.94, P<0.001),$$

$$\text{Winter: } T_{\text{CL}} = 295.6M_b^{-0.035} - 273 \quad (N=5, R^2=0.91, P=0.01),$$

where T_{CL} is in °C and M_b is in g. For subsequent T_{CL} experiments, sliding helox cold exposure was initiated at temperatures 6°C above the allometrically predicted T_{CL} . The initial temperature was then modified for each species, as

Table 1. Mass, summit metabolism and cold tolerance data for summer- and winter-acclimatized small birds

Species (N)	M_b (g)	M_{sum} (W)	T_{CL} (°C)	t_{hypo} (min)
Summer acclimatized				
Downy woodpecker <i>Picoides pubescens</i> (4)	25.8±0.8	2.59±0.36	2.3±3.9	64.8±31.5
Hairy woodpecker <i>P. villosus</i> (2)	62.6	4.24	-6.4	99
Eastern wood-pewee <i>Contopus virens</i> (5)	13.7±1.1	1.23±0.13	4.4±1.7	54.8±11.4
Eastern kingbird <i>Tyrannus tyrannus</i> (5)	40.5±4.5	2.95±0.36	-1.2±2.1	61.8±20.4
Bell's vireo <i>Vireo bellii</i> (6)	9.2±0.5	0.85±0.11	10.0±2.1	41.3±8.6
Warbling vireo <i>V. gilvus</i> (6)	13.4±0.4	1.39±0.19	4.8±1.6	43.7±12.2
Black-capped chickadee <i>Poecile atricapillus</i> (5)	13.2±1.3	1.47±0.08	4.7±1.8	54.6±13.4
White-breasted nuthatch <i>Sitta carolinensis</i> (5)	19.6±1.1	1.63±0.29	7.0±2.8	33.4±9.7
House wren <i>Troglodytes aedon</i> (4)	10.3±0.2	1.30±0.26	6.1±1.0	60.8±8.8
Gray catbird <i>Dumetella carolinensis</i> (7)	34.8±2.3	2.32±0.31	-0.7±2.0	61.4±16.5
Yellow warbler <i>Dendroica petechia</i> (5)	9.3±0.3	0.97±0.11	8.4±2.4	55.0±23.1
Northern cardinal <i>Cardinalis cardinalis</i> (5)	41.4±1.4	2.71±0.19	-2.7±2.4	68.6±18.9
Rose-breasted grosbeak <i>Pheucticus ludovicianus</i> (5)	40.0±3.0	2.50±0.45	-2.9±2.3	65.4±22.3
Indigo bunting <i>Passerina cyanea</i> (5)	13.9±1.1	1.30±0.11	6.1±1.9	49.0±12.1
Chipping sparrow <i>Spizella passerina</i> (5)	11.7±0.5	1.20±0.11	5.9±3.8	54.8±21.2
Field sparrow <i>S. pusilla</i> (5)	12.5±1.4	1.52±0.34	1.0±5.2	58.4±14.2
Orchard oriole <i>Icterus spurious</i> (5)	20.4±1.0	1.68±0.18	4.2±1.8	51.0±11.1
Baltimore oriole <i>I. galbula</i> (5)	31.0±1.2	2.51±0.11	-2.4±2.1	82.6±25.0
House finch <i>Carpodacus mexicanus</i> (5)	21.3±0.4	2.02±0.17	-6.0±2.2	85.2±33.6
American goldfinch <i>Carduelis tristis</i> (8)	12.1±1.1	1.39±0.16	1.9±2.4	73.3±15.2
House sparrow <i>Passer domesticus</i> (6)	26.8±1.7	2.82±0.26	-5.5±1.7	93.5±26.1
Winter acclimatized				
Downy woodpecker <i>P. villosus</i> (6)	26.0±1.1	2.41±0.43	-7.1±2.7	71.3±40.8
Horned lark <i>Eremophila alpestris</i> (4)	35.4±2.7	3.42±0.16	-14.2±0.2 ^a	170.3±11.8 ^a
Black-capped chickadee <i>Poecile atricapillus</i> (12)	13.0±0.9	2.01±0.26	-7.7±1.8	69.6±23.6
Red-breasted nuthatch <i>Sitta canadensis</i> (3)	10.5±0.7	1.56±0.04	-2.5±3.1	65.7±22.4
White-breasted nuthatch <i>Sitta carolinensis</i> (5)	21.8±0.7	2.08±0.19	-3.5±2.2	39.8±6.7
American tree sparrow <i>Spizella arborea</i> (4)	18.0±0.8	2.25±0.16	-11.8±2.0	104.3±16.1
Dark-eyed junco <i>Junco hyemalis</i> (6)	19.8±1.0	2.21±0.19	-10.0±2.4	68.2±22.6
Northern cardinal <i>Cardinalis cardinalis</i> (4)	48.3±3.6	3.65±0.06	-11.7±0.4 ^a	105.3±17.0 ^a
House finch <i>Carpodacus mexicanus</i> (7)	21.0±0.8	2.19±0.27	-9.7±1.7	84.3±30.8
American goldfinch <i>Carduelis tristis</i> (11)	13.7±0.6	1.87±0.16	-8.4±1.9	87.1±19.7
House sparrow <i>Passer domesticus</i> (11)	27.1±1.4	3.13±0.19	-8.9±2.3	69.8±34.7

M_b , body mass; M_{sum} , summit metabolism; T_{CL} , cold limit temperature; t_{hypo} , time to reach hypothermia.

^aOnly one of three horned larks became hypothermic, so -14.2°C underestimates actual T_{CL} and 170.3 min underestimates t_{hypo} . Similarly, one northern cardinal did not become hypothermic so T_{CL} and t_{hypo} are slightly underestimated.

needed, so that hypothermia did not occur too rapidly (<45 min) or too slowly (>2 h) for comparative purposes.

In the current study, we measured T_{CL} concurrently with M_{sum} determination on individual birds. We measured summit metabolic rate by open-circuit respirometry using a sliding cold exposure in helox (Swanson et al., 1996). Briefly, we placed birds into 1.9 l or 3.8 l paint cans (depending on body size), with the inner surface painted flat black to provide emissivities near 1.0, which served as metabolic chambers. Mean effective volumes of these chambers, calculated according to Bartholomew et al. (1981), were 1917 ml and 4688 ml for the 1.9 l and 3.8 l chambers, respectively. We achieved temperature control within metabolic chambers by immersing them into a bath of water and propylene glycol (Forma Scientific Model 2095; Marietta, OH, USA), which regulated chamber temperature to ±0.5°C. Prior to immersion, we

flushed the chamber for at least 5 min with helox to replace air with helox. We maintained flow rates of dry, CO₂-free, helox at 1010–1030 ml min⁻¹ over the course of the experiments using a Cole-Parmer Precision Rotameter (Model FM082-03ST; Chicago, IL, USA), previously calibrated to ±1% accuracy. We measured fractional oxygen content in excurrent gas leaving the chamber using an Ametek S-3A oxygen analyzer (Pittsburgh, PA, USA). We recorded fractional oxygen content every 60 s over the test period and computed oxygen consumption according to the instantaneous equations of Bartholomew et al. (1981). We then calculated consecutive 10 min means for oxygen consumption rates over the test period (1–10, 2–11, etc.) and considered the highest 10 min mean, excluding the initial 10 min of measurements), as M_{sum} (Dawson and Smith, 1986). We corrected all values for oxygen consumption to STPD and converted oxygen consumption to

metabolic rates (in W) by assuming an energy equivalent of $20.1 \text{ J ml}^{-1} \text{ O}_2$.

During cold exposure treatments, we exposed individual birds to a declining series of temperatures in helox (3°C every 20 min after 25 min at the initial test temperature) until a gradual decrease in oxygen consumption indicative of hypothermia occurred. These conditions have been shown to elicit M_{sum} in birds (Dutenhoffer and Swanson, 1996; Swanson et al., 1996). For a few individuals in winter (three horned larks *Eremophila alpestris*, two northern cardinals *Cardinalis cardinalis*), birds did not become hypothermic after 3 h of cold exposure reaching the lowest temperatures the bath was capable of attaining (approximately -17.5°C), so it is not certain that these individuals attained M_{sum} and they did not reach T_{CL} . However, oxygen consumption in these individuals was essentially constant over at least the last hour of cold exposure, despite declining temperatures in helox, so it is likely that birds were very close to M_{sum} . In addition, since some individuals of these species did become hypothermic at similar temperatures, it is likely that these individuals had also approached T_{CL} . Although mean T_{CL} was undoubtedly slightly underestimated for these species, this should make the interspecific $M_{\text{sum}}/T_{\text{CL}}$ relationship conservative, as these two

species had higher M_{sum} than was allometrically predicted (Table 1).

Data analyses

We analyzed the relationship between M_{sum} and T_{CL} both by conventional statistical methods and by phylogenetically independent contrasts (Felsenstein, 1985; Garland et al., 1992). For conventional analyses, we performed least-squares regressions of $\log M_{\text{b}}$ vs $\log M_{\text{sum}}$ and $\log M_{\text{b}}$ vs $\log T_{\text{CL}}$. We then calculated residuals from these allometric equations and performed least-squares regression of residuals of $\log T_{\text{CL}}$ against residuals of $\log M_{\text{sum}}$. While this approach controls for the effects of mass on the $M_{\text{sum}}/T_{\text{CL}}$ relationship, it does not account for possible phylogenetic influence on the relationship.

Consequently, we calculated phylogenetically independent contrasts (PIC) for $\log M_{\text{b}}$, $\log M_{\text{sum}}$ and $\log T_{\text{CL}}$ according to Garland et al. (1992, 1993). Calculation of phylogenetically independent contrasts requires knowledge of tree topology and branch lengths, which we garnered from Sibley and Ahlquist (1990) (Fig. 1). Most species for which we measured M_{sum} in this study either have branch length data provided directly in the study of Sibley and Ahlquist (1990) or are closely related to species that are listed, so that branch lengths can be

determined. We used arbitrary branch lengths of 1.0 in the summer analysis for divergences of chipping (*Spizella passerina*) and field (*S. pusilla*) sparrows and for Baltimore (*Icterus galbula*) and orchard (*I. spurius*) orioles (based, respectively, on divergence distances within *Melospiza* sparrows of 1.3 or less and a divergence distance of 1.2 for orioles and New World blackbirds; Sibley and Ahlquist, 1990). In addition, we used a branch length of 2.8 for the Bell's-warbling vireo divergence, because that is the divergence distance between congeneric blue-headed and white-eyed vireos (Sibley and Ahlquist, 1990). In addition, analyses using PIC are robust to actual branch length variation (Garland et al., 1999), so the few arbitrary branch lengths used in this study are unlikely to influence PIC results. We initially standardized contrasts by dividing by branch lengths, but absolute values of contrasts were potentially correlated with their branch lengths, so branch lengths were log-transformed after first increasing the scale of the entire phylogenetic tree by a factor of 10. This reduced correlations to non-significant levels so that contrasts were weighted equally in subsequent analyses. Standardized contrasts were positivized on M_{b} according to Garland et al. (1992). We then performed least-squares regression through the origin on positivized contrasts of $\log M_{\text{b}}$ vs $\log M_{\text{sum}}$ and on $\log M_{\text{b}}$ vs $\log T_{\text{CL}}$. We calculated residuals from $\log M_{\text{sum}}$ and $\log T_{\text{CL}}$ PIC allometric regressions and performed least-

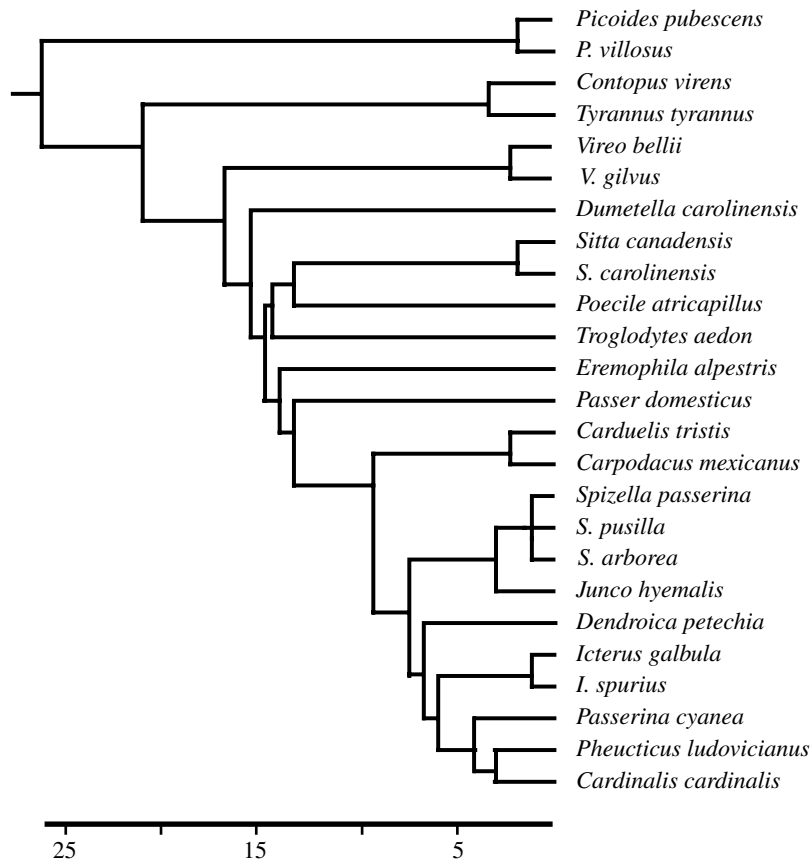


Fig. 1. Phylogeny of bird species used in comparative analyses in this study. Tree topology and branch lengths represent genetic distances (ΔT_{50H} values) from DNA/DNA hybridization data (Sibley and Ahlquist 1990). The total distance from the base node of the tree to the branch tips is 26.3 for the species in this study.

squares regression on residuals of $\log T_{CL}$ contrasts against residuals of $\log M_{sum}$ contrasts to test for phenotypic correlation independent of body mass and phylogeny.

To analyze phylogenetic diversity in the relationship between M_{sum} and T_{CL} , we calculated 95% confidence intervals around allometric regression lines for raw data and PIC regressions for M_{sum} and T_{CL} . We considered values for species (raw data) or for ancestral nodes (PIC) falling outside these confidence intervals as having high or low M_{sum} or T_{CL} (for allometric regressions).

Results

We generated M_{sum} and T_{CL} data for 21 species in summer and 11 species in winter that ranged in body mass from 9.2–62.6 g (Table 1). Mean time to hypothermia (t_{hypo}) for different species ranged from 33 to 99 min in summer, with most values between 45 and 90 min, and from 40–170 min in winter, with most values between 65 and 105 min, so the goal of inducing hypothermia between 45 and 120 min was met for most species (Table 1).

Standard analysis

Least-squares regression yielded significant positive

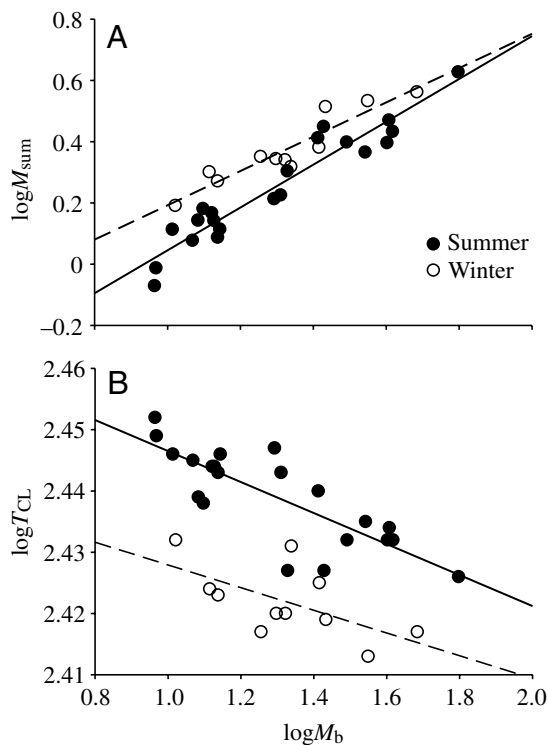


Fig. 2. Regressions of $\log M_{sum}$ (A) and $\log T_{CL}$ (B) against $\log M_b$ in summer (solid line) and winter (broken line). At both seasons, $\log M_{sum}$ was significantly and positively associated with $\log M_b$ and $\log T_{CL}$ was significantly and negatively related to $\log M_b$. Winter equations were significantly elevated for $\log M_{sum}$ and significantly lower for $\log T_{CL}$ than summer equations, suggesting that winter increases in M_{sum} are correlated with decreases in T_{CL} . M_{sum} , W; T_{CL} , °K; M_b , g.

relationships between $\log M_b$ (in g) and $\log M_{sum}$ (in W) for both summer and winter birds (Fig. 2A). Regression equations were:

$$\text{Summer: } \log M_{sum} = -0.65 + 0.70 \log M_b \quad (R^2=0.91, P<0.001)$$

$$\text{Winter: } \log M_{sum} = 0.37 + 0.56 \log M_b \quad (R^2=0.87, P<0.001)$$

Slopes of $\log M_{sum}$ vs $\log M_b$ regressions did not differ significantly between seasons ($F_{1,60}=4.63, P>0.05$), but the winter intercept was significantly higher than the summer intercept ($F_{1,29}=27.83, P<0.001$). Similarly, $\log M_b$ (in g) and $\log T_{CL}$ (in °K) were significantly negatively related in both summer and winter birds (Fig. 2B). Regression equations were:

$$\text{Summer: } \log T_{CL} = 2.47 - 0.025 \log M_b \quad (R^2=0.65, P<0.001)$$

$$\text{Winter: } \log T_{CL} = 2.45 - 0.018 \log M_b \quad (R^2=0.38, P=0.044)$$

Slopes of $\log T_{CL}$ vs $\log M_b$ regressions did not differ significantly between seasons ($F_{1,60}=1.44, P>0.05$), but the winter intercept was significantly lower than the summer intercept ($F_{1,29}=87.99, P<0.001$). Residuals of $\log M_{sum}/\log M_b$ and $\log T_{CL}/\log M_b$ regressions were significantly negatively related in both summer ($R^2=0.34, P=0.006$) and winter ($R^2=0.40, P=0.037$) (Fig. 3).

Phylogenetically independent contrast analysis

Least-squares regression through the origin of phylogenetically independent contrasts of $\log M_{sum}$ against $\log M_b$ yielded significant positive relationships for both summer and winter birds. For summer birds, regression statistics were $b=0.70, R^2=0.66, P<0.001$. Regression statistics for this relationship in winter were $b=0.50, R^2=0.73, P=0.001$. Regressions through the origin for $\log M_b$ and $\log T_{CL}$ (°K) contrasts were significantly negatively related in summer birds and showed a similar non-significant trend for winter birds. Regression statistics for the summer equation were $b=-0.029, R^2=0.29, P=0.012$. Regression statistics for the winter equation

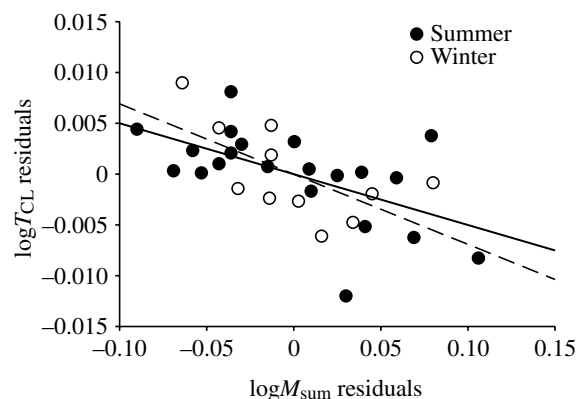


Fig. 3. Residuals from $\log M_{sum}$ vs $\log M_b$ regressions plotted against residuals from $\log T_{CL}$ vs $\log M_b$ regressions. Residuals of $\log M_{sum}$ were significantly and negatively associated with residuals of $\log T_{CL}$ at both seasons, indicating a phenotypic correlation between M_{sum} and T_{CL} .

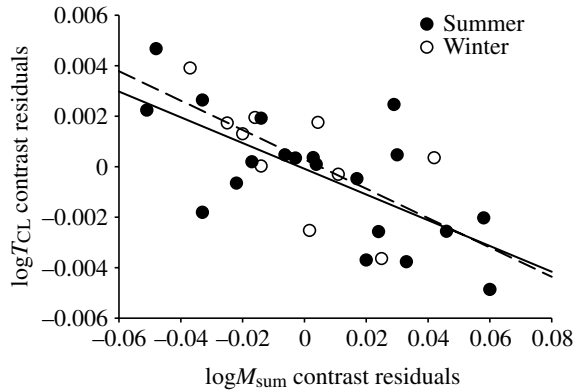


Fig. 4. Residuals from $\log M_{\text{sum}}$ contrasts vs $\log M_b$ contrasts regressions plotted against residuals from $\log T_{\text{CL}}$ contrasts vs $\log M_b$ contrasts regressions. Residuals of $\log M_{\text{sum}}$ contrasts were significantly and negatively associated with residuals of $\log T_{\text{CL}}$ contrasts at both seasons, indicating a phenotypic correlation between M_{sum} and T_{CL} independent of both body mass and phylogeny.

were $b = -0.012$, $R^2 = 0.25$, $P = 0.124$. Residuals from allometric equations for $\log M_{\text{sum}}$ and $\log T_{\text{CL}}$ contrasts were significantly negatively correlated in both summer ($R^2 = 0.47$, $P < 0.001$) and winter ($R^2 = 0.40$, $P = 0.049$) (Fig. 4).

Phylogenetic diversity

Species exhibiting high M_{sum} in summer included downy woodpecker, house wren, black-capped chickadee, house sparrow, American goldfinch and field sparrow (Fig. 5A). Species with low M_{sum} in summer were eastern wood-pewee, Bell's vireo, gray catbird, white-breasted nuthatch, orchard oriole and rose-breasted grosbeak. Those species with high or low M_{sum} also generally showed low or high T_{CL} , respectively. Exceptions included gray catbird and rose-breasted grosbeak, which had low M_{sum} but typical T_{CL} , house wren and black-capped chickadee, which had high M_{sum} but typical T_{CL} , downy woodpecker, which had high M_{sum} but high T_{CL} , and house finch, which had typical M_{sum} but low T_{CL} .

Winter species with high M_{sum} included black-capped chickadee and house sparrow, whereas those with low M_{sum} were downy woodpecker and white-breasted nuthatch; these latter two species also showed high T_{CL} (Fig. 5B). However, even though chickadees and house sparrows had high M_{sum} , their T_{CL} was typical for allometric predictions. American tree sparrows had low T_{CL} , despite exhibiting typical M_{sum} for their body size.

PIC analyses documented ancestral nodes showing high or low M_{sum} or T_{CL} (Fig. 5). For summer analyses, nodes with high M_{sum} included the root node for the entire tree, the vireo node, the house sparrow-sister taxon node, the *Spizella* node, the warbler-oriole/cardinalid node, and the oriole node (Fig. 5A). Nodes with low M_{sum} were the catbird node, the chickadee-nuthatch/wren node, the nuthatch-wren node, the *Spizella*-warbler/oriole/cardinalid node and the oriole-cardinalid node (Fig. 5B). Nodes showing high or low M_{sum}

also generally showed low or high T_{CL} , respectively. Exceptions included the root node and the warbler-oriole/cardinalid node, which had high M_{sum} but typical T_{CL} , the oriole-cardinalid node, which had low M_{sum} but typical T_{CL} , and the woodpecker and nuthatch/wren/chickadee nodes, which had typical M_{sum} but low T_{CL} . The only winter node with high M_{sum} was the house sparrow-finch/sparrow/cardinalid node, but this node showed typical T_{CL} . The only winter node with low M_{sum} was the nuthatch-chickadee node, which also showed high T_{CL} . The nuthatch/chickadee-sister taxon and horned lark-sister taxon nodes both showed low T_{CL} but typical M_{sum} .

Discussion

Cold tolerance and thermogenic capacity were positively correlated in both summer and winter on an interspecific basis for both standard and phylogenetically corrected analyses in this study. This indicates that species with higher thermogenic capacity also showed greater cold tolerance, as measured by lower T_{CL} , suggesting that cold tolerance and thermogenic capacity are functionally linked. These data are consistent with intraspecific data on thermogenic capacity and cold tolerance in small birds. Swanson (2001) measured cold tolerance as shivering endurance under cold stress in black-capped chickadees *Poecile atricapillus*, dark-eyed juncos *Junco hyemalis* and American tree sparrows *Spizella arborea*, all of which showed positive correlations between shivering endurance and thermogenic capacity. Thus, both within and among species comparisons demonstrate a positive correlation between cold tolerance and thermogenic capacity, strongly suggesting a functional link between them.

Such a correlation is also generally consistent with previous data on seasonal acclimatization in small birds. A few birds exhibit seasonal changes in cold tolerance without accompanying seasonal changes in thermogenic capacity, and geographic variation in cold tolerance is not always associated with corresponding variation in thermogenic capacity (Dawson et al., 1983a; Swanson, 1993; Saarela et al., 1995). Such data have cast doubt on the generality of the correlation between thermogenic capacity and cold tolerance. However, most species of small birds do show a significant winter increment of thermogenic capacity that is associated with substantial improvements in capacity to tolerate cold temperatures (Hart, 1962; Swanson, 1990a; Cooper and Swanson, 1994; O'Connor, 1995a; Liknes and Swanson, 1996; Liknes et al., 2002; Cooper, 2002; Arens and Cooper, 2005a). Winter increments of thermogenic capacity documented in these studies range from 16–55%. If thermogenic capacity and cold tolerance are generally elevated in winter relative to summer in small birds, then regression equations of $\log M_{\text{sum}}$ on $\log M_b$ should be elevated, and regression equations of $\log T_{\text{CL}}$ on $\log M_b$ should be lower, in winter compared to summer. Such was indeed the case in this study, as slopes of these regressions did not differ significantly between seasons, but intercepts were

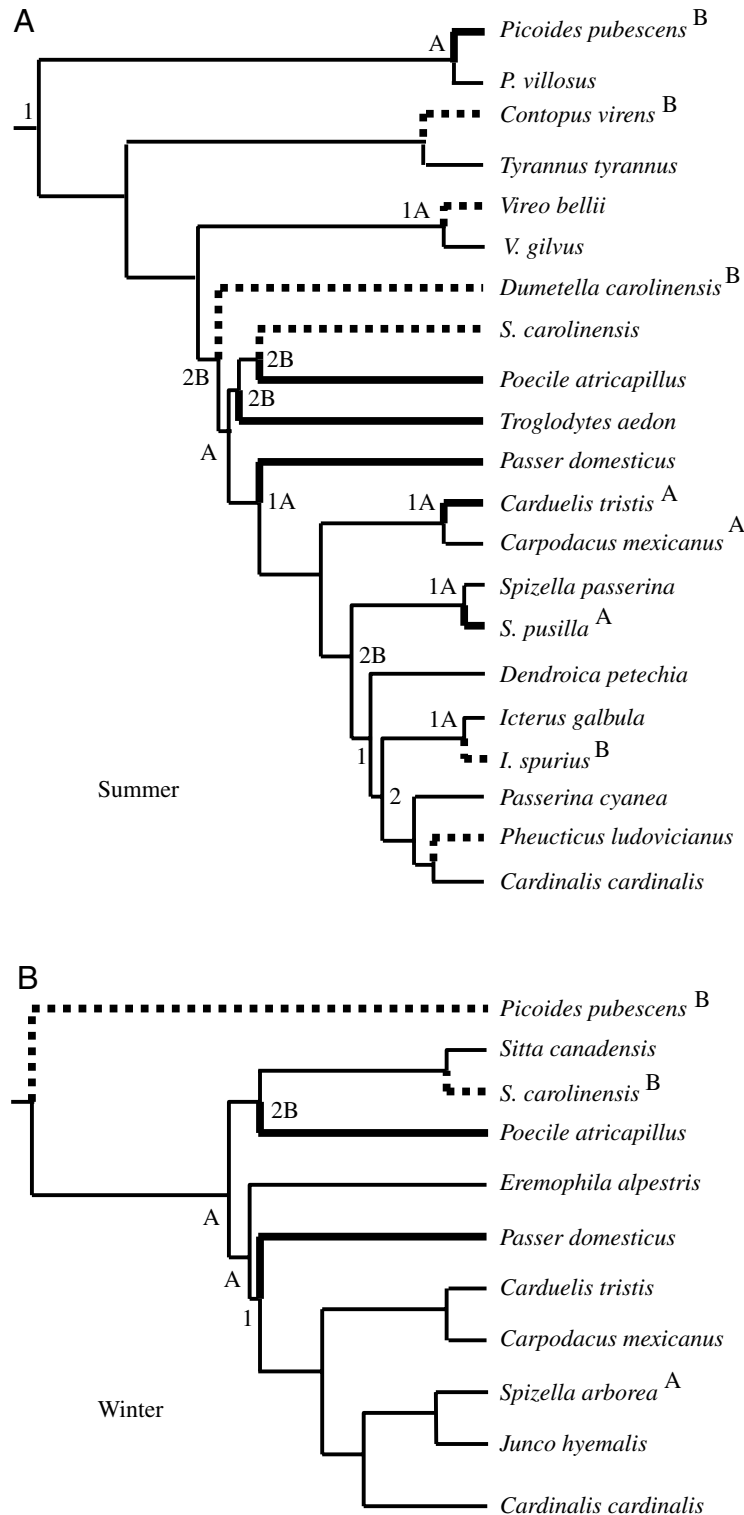


Fig. 5. Occurrence of high (solid bold lines) and low (broken lines) M_{sum} for tips of phylogenies for summer and winter birds in this study. The numbers and letters at the ancestral nodes and branch tips refer to high M_{sum} (1), low M_{sum} (2), low T_{CL} (good cold tolerance; A), high T_{CL} (poor cold tolerance; B). Low and high M_{sum} and T_{CL} were determined from tips or nodes that fell outside of 95% confidence intervals from allometric regressions of $\log M_{sum}$ and $\log T_{CL}$ on $\log M_b$ for both raw data and phylogenetically independent contrasts.

significantly higher for M_{sum} and significantly lower for T_{CL} in winter than in summer. In general, therefore, winter birds had higher thermogenic capacity and tolerated colder temperatures in helox than summer birds. For example, according to the regression equations in this study, a 20 g bird would have a 28.2% higher M_{sum} and would require a helox temperature 6.8°C lower to induce hypothermia in winter relative to summer. The seasonal temperature difference for hypothermia induction in helox substantially underestimates the actual seasonal temperature difference in air, as helox markedly increases thermal conductivity relative to air in small birds (Dawson and Smith, 1986; Swanson, 1993; Cooper, 2002), so seasonal differences in cold tolerance are quite marked for the species in this study.

Thus, winter increment of thermogenic capacity appears to be a common component of seasonal acclimatization in small birds. Taken together, data demonstrating concomitant seasonal variation in cold tolerance and thermogenic capacity and direct demonstration of correlations between cold tolerance and thermogenic capacity, both within and among species, strongly suggest that physiological adjustments promoting increased thermogenic capacity in small birds also promote elevated cold tolerance. This suggests that cold tolerance (i.e. thermogenic endurance) and thermogenic capacity are functionally linked, potentially through variation in muscle mass or by adjustments of mass-specific metabolic intensity or capacity to oxidize fuels, principally fat (Dawson et al., 1983b; Marsh and Dawson, 1989; Swanson, in press). Such a link is consistent with the general vertebrate pattern of coupled variation in endurance and aerobic capacity (Bennett, 1991).

Because metabolic rates (M) in endotherms can be defined by:

$$M = C(T_b - T_a) ,$$

where C is thermal conductance (a net measure of heat transfer between the animal and the environment), T_b is body temperature and T_a is ambient temperature, a link between M_{sum} and T_{CL} is perhaps not surprising. At temperatures eliciting maximum cold-induced metabolic rates in birds, M_{sum} and T_{CL} can potentially be substituted into the above equation, yielding, after rearrangement:

$$T_{CL} = T_b - (M_{sum} / C) ,$$

which suggests that M_{sum} and T_{CL} should be linked (e.g. Bozinovic and Rosenmann, 1989). However, two factors could influence this purported linkage. First, variation in M_{sum} is not the only factor that influences T_{CL} . Concurrent variation in C or T_b could offset any variation in M_{sum} , such that M_{sum} and T_{CL} might not be correlated. In essence, testing for a correlation between M_{sum} and T_{CL} is akin to testing for how much variation in T_{CL} is explained by variation in M_{sum} , rather than by other

factors that affect C or T_b . Second, substituting M_{sum} and T_{CL} into the above equation assumes that T_{CL} always occurs concurrently with M_{sum} , but this is often not the case, as the highest metabolic rates (M_{sum}) during cold exposure treatments, such as those in this study, usually occur well before temperatures eliciting hypothermia (Swanson, 2001). Thus, substituting M_{sum} and T_{CL} into the equation describing metabolic rates in endotherms is probably not strictly appropriate.

R^2 values for regressions of residuals from allometric equations for M_{sum} and T_{CL} ranged from 34–47% in this study, indicating that interspecific variation in thermogenic capacity explained a substantial portion of the interspecific variation in cold tolerance. However, substantial variation in cold tolerance still remains unexplained, which suggests a role for other factors in affecting differences in cold tolerance among species and seasons. Such factors could include differences in insulation, control over thermal conductance, circulatory and ventilatory differences (Swanson, 1990b; Breuer et al., 1995; Arens and Cooper, 2005a,b), or metabolic adjustments promoting shivering endurance without affecting thermogenic capacity (Marsh and Dawson, 1982; Yacoe and Dawson, 1983; Marsh et al., 1990).

Because seasonal acclimatization in birds is largely a metabolic process, with only a minor role played by seasonal changes in insulation (Dawson et al., 1983b; Marsh and Dawson, 1989; Swanson, 1991a), metabolic adjustments should play a prominent role in explaining both seasonal and interspecific variation in cold tolerance. Such metabolic adjustments could include those affecting fuel mobilization and supply to shivering muscles, as well as those promoting preferential use of lipid to fuel shivering (Marsh and Dawson, 1982; Yacoe and Dawson, 1983; Marsh et al., 1990; Swanson, 1991b; O'Connor, 1995b). These adjustments would not necessarily be reflected by increases in thermogenic capacity, but could increase cold tolerance by elevating the percentage of thermogenic capacity that could be sustained for prolonged periods. This model for seasonal variation in cold tolerance was posited by Marsh and Dawson (1989), largely from studies on American goldfinches and house finches. Liknes et al. (2002) termed this model the variable fraction model, because the model contends that it is the fraction of thermogenic capacity that is sustainable which varies seasonally, rather than the thermogenic capacity. In contrast to this model is the variable maximum model (Liknes et al., 2002), which posits that it is thermogenic capacity that varies seasonally. The winter increment of thermogenic capacity, in turn, increases thermogenic endurance in the cold, because as thermogenic capacity increases, the absolute rate of sustainable heat production also increases, even if the fraction of thermogenic capacity that is sustainable remains seasonally constant. Because the data in this study indicate a winter increment of thermogenic capacity and directly document a correlation between thermogenic capacity and cold tolerance in small birds, they are consistent with the variable maximum model. However, it is important to note

that metabolic adjustments promoting maintenance of a higher sustained fraction of thermogenic capacity could further improve cold tolerance, and therefore might help account for some of the unexplained variation in cold tolerance in this study.

Some interesting general trends emerged from analyses of phylogenetic diversity in the relationship between M_{sum} and T_{CL} . For summer analyses, the root node had high M_{sum} , but typical T_{CL} based on allometric predictions, whereas in winter the root node was typical for both parameters. The summer data suggest that ancestral species had high thermogenic capacity, but were relatively poorly insulated, resulting in relatively poor cold tolerance for their metabolic abilities. However, in winter, where taxa not resident in cold climates were absent from the analyses, the root node was typical for both M_{sum} and T_{CL} , suggesting that it is taxa not resident in cold climates that were driving the uncoupling of M_{sum} and T_{CL} from summer analyses. Another factor likely influences this uncoupling, however, and that is the absence of a winter increase in M_{sum} in downy woodpeckers in this study. Because downy woodpeckers had high M_{sum} in summer and low M_{sum} in winter, and woodpeckers were one of the sister taxa at this node, the nodal values were likely influenced by the absence of a seasonal difference in M_{sum} in this species. The lack of a seasonal difference in M_{sum} in downy woodpeckers differs from that previously documented for this species by Liknes and Swanson (1996), where M_{sum} in winter was 52% greater than that in summer. The reason for the difference between these two studies is unknown, but may involve differences in winter weather among years, which can impact metabolic rates in birds (Swanson and Olmstead, 1999).

Another noteworthy finding from summer analyses was that high M_{sum} and low T_{CL} , as well as low M_{sum} and high T_{CL} , occurred in taxa composed solely of migrants, as well as taxa with members wintering in cold climates. This suggests that physiological capacities for heat production or cold tolerance are not the sole determinant of wintering strategy within a taxon. Finally, although deviations from allometric predictions for M_{sum} and T_{CL} were usually coupled for species and for ancestral nodes, this was not always the case. This again suggests that while thermogenic capacity is a prominent factor influencing cold tolerance, there is still room for factors other than thermogenic capacity in establishing differences in cold tolerance among species and seasons.

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