The Relationship of Metabolic Performance and Distribution in Black-Capped and Carolina Chickadees

Jennifer R. Olson[1,*]
Sheldon J. Cooper[2]
David L. Swanson[3]
Michael J. Braun[4]
Joseph B. Williams[1]
[1]Department of Evolution, Ecology and Organismal Biology, Ohio State University, 318 West 12th Avenue, Columbus, Ohio 43210; [2]Department of Biology and Microbiology, University of Wisconsin–Oshkosh, 800 Algoma Boulevard, Oshkosh, Wisconsin 54901-8640; [3]Department of Biology, University of South Dakota, 414 East Clark Street, Vermillion, South Dakota 57069; [4]Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution, 4210 Silver Hill Road, Suitland, Maryland 20746, and Behavior, Ecology, Evolution, and Systematics Program, University of Maryland, College Park, Maryland 20742

Accepted 9/13/2009; Electronically Published 2/3/2010

Online enhancement: color version of figure 1.

ABSTRACT

In endotherms, metabolic performance is associated with a wide array of ecological traits, including species distribution. Researchers have suggested that the northern boundaries of North American passerines are limited by their ability to sustain the high metabolic rates required for thermoregulation. Black-capped chickadees (*Poecile atricapillus*; BC) are year-round residents in most of Canada and the northern half of the United States, whereas Carolina chickadees (*Poecile carolinensis*; CA) are found exclusively in the southeastern United States. These species hybridize along a narrow contact zone that has been moving northward at a rate of about 1.6 km per decade, coincident with warming temperatures in Ohio. The location of the chickadee hybrid zone in Ohio closely matches air temperature isotherms, further suggesting that metabolic rate may correlate with distribution in these species. We tested the hypothesis that distribution patterns of chickadees are linked with their rate of metabolism. For populations of BC and CA chickadees, we measured basal metabolic rates (BMRs) and cold-induced peak metabolic rates from areas that differ in winter temperatures and supplemented this information with data from other studies. Although our findings suggest a general relationship between lower air temperatures and higher metabolic rate among black-capped chickadee populations, this trend was not robust across all locations. There was no significant relationship between lower air temperatures and metabolism in Carolina chickadees. Within Ohio, hybrids had a significantly higher mass-corrected BMR than either parental species. We suggest that the mtDNA-nDNA mismatch of hybrids may produce less efficient mitochondrial protein complexes, which in turn affects the efficiency of ATP production, thereby increasing rate of oxygen consumption to meet ATP demands.

* Corresponding author; e-mail: olson.133@osu.edu.

Physiological and Biochemical Zoology 83(2):263–275. 2010. 1522-2152/2010/8302-8259$15.00
DOI: 10.1086/648395

Introduction

Metabolic performance is a fundamental physiological attribute that correlates with many aspects of a species' ecology, including its distribution, survival, behavior, and life history (Aschoff and Pohl 1970; Weathers 1979; White et al. 2007; Wiersma et al. 2007*b*). Two indices are commonly used to assess metabolic performance: (1) basal metabolic rate (BMR), the minimum metabolic rate of a quiescent, postabsorptive animal, in its thermal neutral zone and rest phase (McNab 1997); and (2) peak metabolic rate (PMR), the maximum rate of energy expenditure, often achieved by exposing birds to cold in metabolic chambers (Wiersma et al. 2007*a*). Cold-induced PMR defines the maximum heat generating capacity of an endotherm and correlates positively with endurance of cold (Swanson 2001). Because the BMR makes up 25%–40% of a bird's field metabolic rate (Bryant 1997) and often correlates with field metabolism (Daan et al. 1991; White and Seymour 2004), it has been used as a proxy for energy expenditure in the wild. Unlike the BMR, which is largely a function of central organs (e.g., brain, heart, kidneys, and gut; Taigen 1983; Rolfe and Brown 1997; Hoppeler and Weibel 1998), heat generation in birds during cold exposure relies on shivering by skeletal muscles (Bennett 1991; Weibel et al. 2004). Although within-species comparisons of BMR and PMR often show a positive correlation (Hinds and Rice-Warner 1992; Dutenhoffer and Swanson 1996; Likenes and Swanson 1996; Rezende et al. 2002; Wiersma et al. 2007*a*), some studies have shown that these two variables are unrelated (Koteja 1987; Vézina et al. 2006).

The impact of metabolic performance on the distribution patterns of birds remains controversial (Root 1988*b*, 1989; Castro 1989; Repasky 1991; Canterbury 2002). In North America, there is evidence that the northern limits of some bird species are correlated with metabolic performance. Root (1988*b*) found

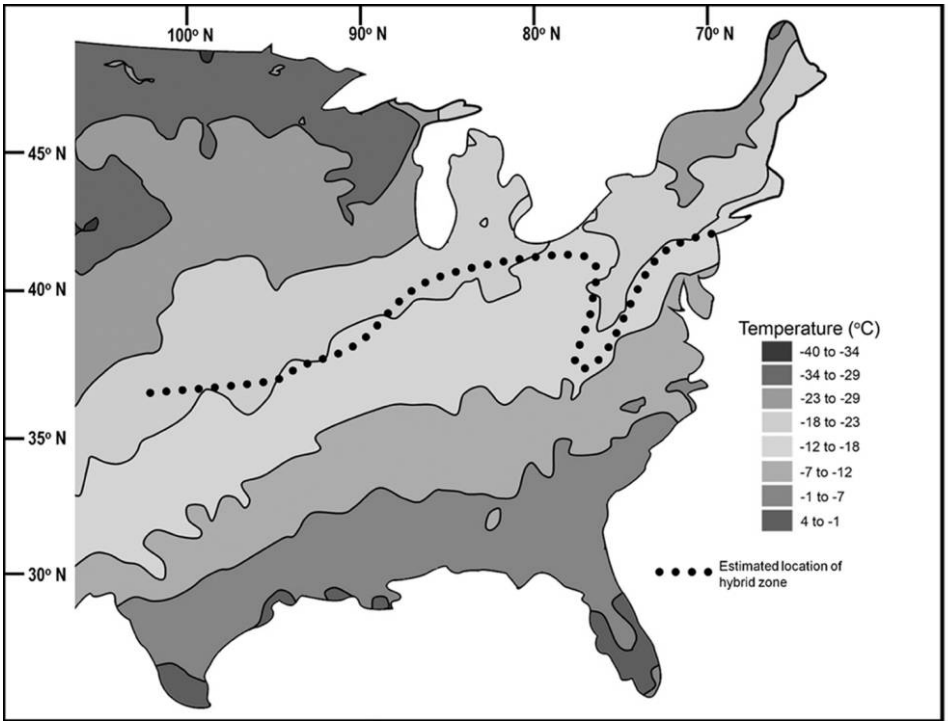

Figure 1. Position of the chickadee hybrid zone in relation to mean minimum winter temperatures. We approximated zone position from North American Breeding Bird Survey data (http://www.mbr-pwrc.usgs.gov/bbs/bbs.html); Tanner 1952; Brewer 1963; Rising 1968; Robbins et al. 1986; Grubb et al. 1994; and Sattler and Braun 2000. Isotherms are based on the mean minimum winter temperature as depicted in *The National Atlas of the United States* (U.S. Geological Survey 1970). A color version of this figure is available in the online edition of the *Physiological and Biochemical Zoology*.

that the northern boundary of 60% of birds wintering in North America coincided with isotherms of minimum daily January temperature and concluded that winter distributions are limited to areas where the birds do not need to raise their metabolic rate more than 2.5 times BMR. Some investigators have challenged Root's analyses and conclusions, rendering the accuracy of her predictions uncertain (Castro 1989; Repasky 1991; Canterbury 2002).

Variation in metabolic performance across populations of the same species, especially those with a large geographic range that transects multiple thermal environments, has not been addressed (Root 1988*a*; Swanson, forthcoming). Such studies could provide insights into the role of metabolism in defining distributional limits of species and insights into how the local environment impacts levels of adjustment of metabolic rate. One might surmise that individuals of the same species inhabiting generally warmer areas will have a lower BMR and PMR than birds that inhabit colder climes—the local adjustment hypothesis. Hence, birds of the same species that experience different winter isotherms might be expected to show corresponding adjustments to BMR and/or PMR.

North American chickadees (family: Paridae) provide a unique system for exploring possible relationships between metabolic performance and distribution. Black-capped chickadees (BC; *Poecile atricapillus*) are year-round residents in most of Canada and the northern half of the United States, whereas Carolina chickadees (CA; *Poecile carolinensis*) are found exclusively in the southeastern United States. The ranges of these two species meet in Kansas eastward to New Jersey where they form a narrow band of hybrids (Ohio: Bronson et al. 2005; Pennsylvania: Reudink et al. 2007; Missouri: Sawaya 1990; Virginia and West Virginia: Sattler and Braun 2000). The northern limit of CA chickadees is associated with an isotherm at −6.7°C (mean minimum January temperature; Repasky 1991), and BC chickadees live as far north as the −26.5°C isotherm (Canada Department of Energy, Mines and Resources 1974; Smith 1993).

Variation in thermal environments suggests that ambient temperature (T_a) is associated with the distribution of BC and CA chickadees (Fig. 1). Unlike a number of other hybrid zones, the contact zone between BC and CA chickadees includes southward extensions of BC chickadees into upper elevations of the Appalachian Mountains as well as extensions into Illinois, Missouri, and Kansas; each interdigitation southward occurs in a region with colder winter climate than other regions at similar latitudes (U.S. Geological Survey 1970). In addition, as ambient temperatures have warmed, populations of CA and BC chickadees have moved northward. Over the last half-century, the mean daily January temperature in Ohio has warmed by approximately 4°C (NOAA 2008). Coincidentally, the contact zone between BC and CA chickadees in Ohio has been moving northward at a rate of approximately 1.6 km per decade (Whea-

ton 1882, Trautman 1940; Grubb et al. 1994; Bronson et al. 2003*a*, 2003*b*).

We examined metabolic performance of five populations of BC chickadees from Alaska, South Dakota, Wisconsin, Ohio, and New York and two populations of CA chickadees from Ohio and Tennessee to test the hypothesis that distribution is related to metabolic performance. In cold temperate climates, winter-acclimatized birds increase their BMR, PMR, and cold tolerance relative to summer-acclimatized birds, in response to the increased energetic demands of thermoregulation (Dawson and Marsh 1989; Swanson 1990; Cooper and Swanson 1994). Using strong inference (Platt 1964), we envisioned several non–mutually exclusive hypotheses that could describe the relationship between BMR, PMR, and latitude in these species. If chickadees optimize their metabolism for the local environment, birds living at higher latitudes should have a higher BMR and PMR compared with conspecifics at lower latitudes. Alternatively, chickadees may not exhibit any latitudinal variation in metabolism.

Hybridization between species of chickadees could impact metabolic performance of offspring, which in turn may affect the position and northward movement of the hybrid zone. Oxidative phosphorylation and oxygen consumption of endotherms are largely a function of their mitochondria (Pon and Schon 2001; Weibel and Hoppeler 2005), and protein complexes within the mitochondria consist of polypeptides encoded by both nuclear and mitochondrial DNA. Because the mtDNA is maternally inherited, hybrid chickadees will have polypeptides derived from both the BC and CA genomes. If mitochondrial genes have diverged in tandem with mitochondrial proteins encoded by the nuclear genome, one might predict that ATP production of hybrids would be less efficient than that parent species, perhaps as a result of increased proton leak or less efficient metabolic enzyme activity (Dobzhansky 1936; Stuart et al. 1999; Harper et al. 2002; Ellison and Burton 2008). Assuming that muscle mass is not affected by hybridization, we hypothesized that hybrids would have a higher BMR than either parental population due to greater oxygen consumption needed to overcome inefficiencies in ATP production. Assuming that maximum oxygen consumption is limited by the rate of ATP synthesis, we also predicted that hybrids would have a lower PMR than either parental population if hybrids do not compensate for this inefficiency through other physiological means (e.g., higher muscle mitochondrial density or increased activity of metabolic enzymes).

Material and Methods

Capture of Birds

We trapped 181 chickadees (BC, CA, and hybrids) in Ashland County, Ohio (40°51′N, 82°10′W), during three winters from December 2003 through February 2006. Traps were located in 26 privately owned woodlots along a 40-km (north-south) × 5-km (east-west) transect that encompassed the hybrid zone between chickadee species as determined by Bronson et al. (2003*a*). In addition, we captured 6 BC in Chagrin Falls, Ohio (41°22′N, 81°22′W), 12 BC in Oshkosh, Wisconsin (44°01′N, 88°33′W), and 15 CA in Paris, Tennessee (36°18′N, 88°19′W) using mist nets during February 2006. Birds from Chagrin Falls were not used for metabolic measurements and were incorporated for genetic analyses only. David Swanson measured BMR and PMR of BC in South Dakota ($n = 47$), and S. Chaplin provided data for BMR for New York BC ($n = 10$; Chaplin 1974).

We housed birds in 30 × 25.5 × 35.5-cm wire mesh cages and provided them with sunflower seeds, mealworms, and water ad lib. Cages were kept in a university-approved animal housing room at ambient indoor temperatures with lights turned on during daylight hours (9L : 15D). We made all metabolic measurements within a week of capture. Experiments were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee (protocol 2004A0065).

Species Identification

Morphological similarity between BC, CA, and hybrids, along with high levels of backcrossing, make species recognition difficult in regions where BC and CA potentially coexist (Sattler and Braun 2000). Based on amplified fragment length polymorphism (AFLP) analysis (Vos et al. 1995), with modifications proposed by Kingston and Rosel (2004), we designated birds as BC, CA, or hybrid. At the time of capture, we punctured the bird's brachial vein using a 25-gauge needle, collected blood in a heparinized capillary tube, placed the sample in lysis buffer (100 mM Tris at pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; Longmire et al. 1988), and stored it at 4°C. We extracted DNA from samples using the methods of Bronson et al. (2003*a*). We digested extracted DNA from blood samples with restriction enzymes EcoRI and TaqI, followed by two rounds of PCR (preselective and selective) to both amplify fragments and fluorescently label them for detection. Fragments were sized using GeneMapper, version 3.7 (Applied Biosystems, Foster City, CA), and we assigned individuals to populations using STRUCTURE, version 2.1 (Pritchard et al. 2000). In addition to the birds above, we also analyzed extracted DNA from 18 CA chickadees previously captured in Lawrence County, OH (38°43′N, 82°34′W; Sattler and Braun 2000) for inclusion as an additional parental population. We analyzed 245 loci from 218 individuals using 100,000 estimation steps after 50,000 burn-in steps and each value of K (the number of clusters) from 1 to 5. We achieved the most logical population structure using a K of 2, and we classified birds as hybrids if they were assigned a 25%–75% probability of belonging to either parental species. We also used restriction fragment length polymorphism to determine mitochondrial haplotypes of chickadees from Ohio, Wisconsin, and Tennessee, using methods described by Reudink et al. (2006).

Measurements of Basal Metabolic Rate

Birds collected in Wisconsin, Ohio, and Tennessee were transported to the lab for measurements of metabolism. For mea-

surements of BMR, we removed food from birds 2 hrs before measurements to assure that they were postabsorptive. Then, at 2000 hours we placed birds in 1-L stainless steel chambers lined with dark brown Teflon to reduce reflective radiation and minimize adherence of water molecules to the chamber (Porter 1969). Each chamber contained a wire-mesh platform and a plastic perch above a layer of mineral oil to capture feces, thus eliminating them as a source of water vapor. Rubber gaskets rendered the lids airtight and excluded light. We placed four chambers into a temperature-controlled cabinet maintained at 32°C, a temperature within the thermal neutral zone of these species (Rising and Hudson 1974), using a Peltier device (Sable Systems Pelt 4). To monitor T_a within the chambers, we inserted 30-gauge copper-constantan thermocouples into each chamber. Compressed air was directed through a column of Drierite to remove water and then through mass flow controllers (Tylan/Mykrolis, Chaska, MN) that we previously calibrated using a glass bubble meter (500 mL; Levy 1964), and the air was then sent into the chamber. An automated system of solenoids sequentially sampled the outflow air from each chamber. Excurrent air passed through a Dew Prime II hygrometer to measure dew point (EdgeTech, Marlborough, MA), through tubes containing silica gel, ascarite, and another layer of silica gel to remove water and CO_2, and then through an Ametek S3AII oxygen analyzer to measure fractional concentration of oxygen. We directed outputs from the oxygen analyzer, dew point hygrometer, and thermocouples to a CR23X micrologger (Campbell Scientific, Logan, UT) with a 1-min sampling rate. Data from each chamber were recorded for 12 min before the solenoid system switched to the next chamber; we continued measurements throughout the night. Mass (± 0.1 g) and cloacal temperature (± 0.1°C) of each bird was recorded before and after each trial. We averaged oxygen consumption over 10-min intervals during the sampling period and selected the lowest constant value for BMR. We calculated rate of oxygen consumption, $\dot{V}O_2$, using equation (4) from Hill (1972).

Measurements of Peak Metabolic Rate

One to 2 d after measurement of BMR, we measured PMR between 0800 and 1800 hours. Two hours before measurements, we removed food to assure that birds were postabsorptive. We measured PMR using a system similar to that for BMR, except that the temperature of the chamber was controlled by a mixture of antifreeze circulating in copper coils that surrounded the chamber, with the entire assembly insulated by Styrofoam. The temperature of the antifreeze was controlled by a refrigerated water bath (NESLAB RTE-140, Pittsburgh, PA). Incurrent air was a mixture of 79% helium and 21% oxygen (heliox; Rosenmann and Morrison 1974). We set the initial water bath temperature at 32°C, and birds were allowed a minimum of 30 min to adjust to the chamber before we began decreasing T_a by about 1.0°C per min. Measurements terminated when oxygen consumption no longer increased with a decrease in chamber temperature (Swanson et al. 1996). We recorded bird mass (± 0.1 g) and cloacal temperature (± 0.1°C) before and after each trial.

For PMR, we calculated instantaneous rates of oxygen consumption according to the equations of Bartholomew et al. 1981 and averaged values over 10-min intervals. We took the highest 10-min mean over the entire test period as PMR. We defined cold tolerance as the temperature at the cold limit (T_{cl}; Saarela et al. 1995), which was the temperature at which a bird achieved PMR during cold trials, and used T_{cl} as a measure of thermal endurance.

Data from the Literature: BMR and PMR

Oxygen consumption of South Dakota and New York chickadees was measured as described by Swanson et al. (1996) and Chaplin (1974), respectively. We obtained BMR measurements from two additional studies on chickadee metabolism. We averaged the eight data points from Figure 1 of Grossman and West (1977), which represented measurements of BMR on BC chickadees from Fairbanks, Alaska (in the birds' thermoneutral zone). We also obtained approximations of BMR for Ohio BC (Ashtabula County, OH) and CA chickadees (Hamilton County, OH) from Table 4 of Munzinger (1974). Because these values represent average nocturnal oxygen consumption from 24-h trials that were conducted at a lower temperature than our measurements (25° vs. 32°C), these data were included in figures for comparison but were not used in any statistical analyses.

Statistical Analyses

We analyzed data from our measurements both within and across species to separate the influence of thermal environment and genetic identity on metabolic rate. In order to determine how metabolism varied within species, we compared whole-organism metabolic rates across the five populations of BC chickadees (Alaska, Wisconsin, South Dakota, New York, and Ohio) and across the two populations of CA chickadees (Ohio and Tennessee) using an ANOVA. Hybrids were not included in these analyses. We then evaluated the whole-organism metabolic rates of chickadees across the hybrid zone in Ohio.

In order to control for the effect of body size on metabolic rate (King and Farner 1961; Lasiewski and Dawson 1967; Weibel and Hoppeler 2005), we used an ANCOVA to examine metabolic rate across populations, using population or species as a fixed factor and mass as a covariate (see Packard and Boardman 1988, 1999; Hayes 2001). Data presented in figures are least square means $\pm$ SE when controlling for mass. As with whole-organism metabolism, we compared BC and CA chickadees across populations and BC, CA, and hybrid chickadees across the OH hybrid zone. When applicable, we employed Sidak post hoc comparisons to identify significant differences between populations. We performed statistical analyses using SPSS 15.0, and all mean values are reported ± 1 SE; $P < 0.05$ was chosen as the lowest acceptable level of significance.

Results

Identification of Individuals to Species

In 1994, Bronson sampled chickadees along a north-south transect in Ohio across the putative boundary between BC and CA chickadees (Bronson et al. 2003*a*). Using five enzyme-probe markers, these authors identified a band of hybrids approximately 15 km wide that ran from 40°46′3″N to 40°55′46″N, as judged by visual inspection of their graphs. We extended our sampling region both north and south of that used by Bronson et al. (2003*a*) and, using AFLP analysis, classified all birds that we measured as BC, CA, or hybrid. The genetic identities of our samples are displayed in Figure 2.

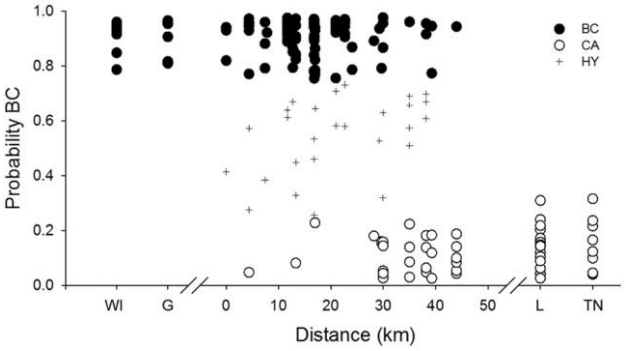

Figure 2. Genetic identity of Ohio birds along a transect of Ashland County, Ohio ($n = 174$). Distance 0 on the *X*-axis is the northernmost woodlot sampled, located at 41°6′N. Distance 40 on the *X*-axis is equivalent to 40 km south of this woodlot. Birds assigned a probability >75% of being BC were labeled BC (*filled circles*), those of probability 25%–75% are hybrid (*crosses*), and those of probability <25% are CA (*open circles*). The genetic identities of samples from Wisconsin (WI; $n = 10$) and Tennessee (TN; $n = 10$) are also included for reference, as well as samples from extreme northern and southern Ohio (G = Geauga County, 41°22′N, $n = 6$; L = Lawrence County, 38°43′N, $n = 18$). The additional Ohio samples are included for genetic reference only and are not incorporated in metabolic analyses.

Body Mass and Metabolic Rate

Body mass of CA chickadees in Tennessee did not differ significantly from conspecifics in Ohio (Table 1; $P < 0.8$). However, among BC chickadees, there was a significant effect of location on body mass ($F_{4,101} = 29.0$, $P < 0.001$), and post hoc analyses indicated that BC chickadees in South Dakota weighed more than those from Wisconsin, New York, or Ohio ($P < 0.008$ for all comparisons) but not more than BC chickadees from Alaska ($P > 0.9$). Within Ohio, mass was significantly different between groups ($F_{2,43} = 7.9$, $P = 0.001$): BC chickadees weighed the most, CAs weighed the least, and hybrids were intermediate. Post hoc tests revealed that hybrid mass did not differ significantly from either parental species ($P > 0.05$ for both).

Within each species, BMR increased with mass (Fig. 3). For CA chickadees, the relationship between BMR and mass was described as $\log \text{BMR (W)} = -1.42 + 0.77 \log \text{mass (g)}$ ($R^2 = 0.22$, $P = 0.02$), whereas for BC chickadees, it was $\log \text{BMR (W)} = -1.98 + 1.35 \log \text{mass (g)}$ ($R^2 = 0.45$, $P < 0.001$). Slopes and intercepts of equations for BMR did not differ significantly between species.

For CA chickadees, $\log \text{PMR (W)} = -1.19 + 1.34 \log \text{mass (g)}$ ($R^2 = 0.33$, $P = 0.004$), and for BC chickadees, $\log \text{PMR (W)} = -0.99 + 1.12 \log \text{mass (g)}$ ($R^2 = 0.52$, $P < 0.001$). Slopes and intercepts of PMR equations did not differ significantly between species.

T_a *and Metabolic Rate across Locations*

With data pooled for BC and CA, we found that whole-organism metabolism was generally related to mean minimum January temperature for 1997–2006 (Fig. 4; BMR: $R^2 = 0.61$, $P < 0.001$; PMR: $R^2 = 0.42$, $P < 0.001$). The relationships between metabolic rate and temperature are as follows: BMR (W) $= 0.191 - 0.009\ T_a$ (°C); PMR (W) $= 0.942 - 0.092\ T_a$ (°C). When examining only populations of black-capped chickadees, this relationship remained significant: BMR (W) $= 0.191 - 0.009\ T_a$ (°C), $R^2 = 0.54$, $P < 0.001$; PMR (W) $= 0.821 - 0.107\ T_a$ (°C), $R^2 = 0.24$, $P < 0.001$; Fig. 3). However, there was no significant relationship between metabolic rate and temperature in Carolina chickadees ($P > 0.7$ for BMR and PMR). By removing the Alaska birds from our statistical analyses, we were able to explore this correlation among BC populations sampled only in the lower 48 states, and we found that the relationship between metabolic rate and temperature was highly significant: BMR (W) $= 0.175 - 0.012\ T_a$ (°C), $R^2 = 0.22$, $P < 0.001$.

Comparison of BMR across Species

Mean BMR of all BC was significantly greater than the mean BMR of all CA chickadees ($F_{1,111} = 37.7$, $P < 0.001$). In an ANCOVA, there was no significant difference in the BMR of BC or CA chickadees after including mass as a covariate.

Comparison of PMR across Species

BC chickadees had a mean PMR of 1.86 ± 0.04 W, and CA chickadees 1.29 ± 0.03 W. Whole-organism PMR was significantly higher in BC chickadees than in CA chickadees ($F_{1,99} = 53.6$, $P < 0.001$), but this difference disappeared when we included mass as a covariate.

Comparison of BMR within Species

We evaluated BMR within species by comparing populations of the same species from different locations (Fig. 5*A*). For CA chickadees, BMR in Tennessee birds did not differ significantly from those in Ohio. We obtained the same results when we included mass as a covariate (see Table 1 for values). In contrast, there were significant differences for whole-organism BMR among BC chickadee populations ($F_{4,84} = 40.8$, $P < 0.001$), although more northerly populations did not consistently have higher BMRs. Alaskan BC had a significantly higher BMR than

Table 1: Mean metabolic data by location

Chickadee Species and Location	Latitude	T_{min} (°C)[a]	Mass (g)[b]	n_{Mass}	BMR (W)	n_{BMR}	PMR (W)	n_{PMR}	T_{cl} (°C)	n_{Tcl}	Reference
Black-capped:											
Alaska	64°54′N	−27.7	12.6 ± .08	8	.43 ± .02	8					Grossman and West 1977
Wisconsin	44°00′N	−11.3	11.1 ± .24	13	.27 ± .01	13	1.5 ± .06	11	−2.3 ± 1.8	11	This study
South Dakota	42°47′N	−10.6	12.9 ± .15	47	.32 ± .01	31	2.1 ± .03	47			This study
New York	42°41′N	−7.9	11.8 ± .25	10	.30 ± .01	10					Chaplin 1974
Ohio (Ashtabula County)	41°52′N	−7.9	11.1		.26 ± .02	4					Munzinger 1974
Ohio (Ashland County)	40°51′N	−6.9	10.8 ± .17	28	.25 ± .006	27	1.5 ± .05	20	6.6 ± 1.6	18	This study
Hybrid:											
Ohio (Ashland County)	40°51′N	−6.9	10.3 ± .48	7	.26 ± .01	7	1.6 ± .05	4	2.9 ± 1.2	3	This study
Carolina:											
Ohio (Ashland County)	40°51′N	−6.9	9.5 ± .18	11	.21 ± .01	9	1.3 ± .05	10	8.1 ± 1.9	10	This study
Ohio (Hamilton County)	39°30′N	−4.7	9.9		.24 ± .01	4					Munzinger 1974
Tennessee	36°18′N	−2.4	9.4 ± .14	16	.21 ± .01	15	1.3 ± .04	13	5.6 ± 1.2	13	This study

Note. BMR = basal metabolic rate; PMR = peak metabolic rate; T_{cl} = temperature at the cold limit.

[a] Minimun daily temperature in January averaged over 10 yr (1997–2006).

[b] Mass and metabolic measurement are reported ±1 SE.

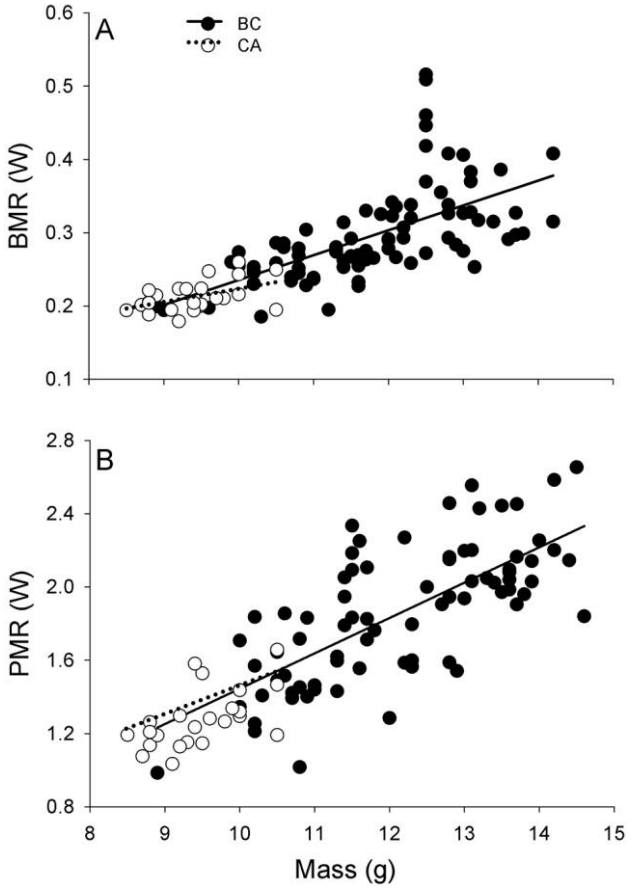

Figure 3. The relationship of basal metabolic rate (BMR; *A*) and peak metabolic rate (PMR; *B*) to mass in black-capped (BC; *filled circles*) and Carolina (CA; *open circles*) chickadees. Linear regression lines are plotted solid for BC and dotted for CA ($P < 0.001$ for both). Sample sizes: BC BMR $n = 71$, CA BMR $n = 24$, BC PMR $n = 77$, and CA PMR $n = 22$.

BC from any other location ($P < 0.001$ for all comparisons). South Dakota BC had a higher BMR than both Wisconsin and Ohio ($P < 0.001$ for both comparisons). Location was also significantly related to BMR in BC after including mass as a covariate ($F_{4,83} = 23.6$, $P < 0.001$). Again, post hoc tests revealed that Alaskan birds had a significantly higher mass-corrected BMR than birds from any other location ($P < 0.001$ for all comparisons). Among the remaining groups, New York and Wisconsin BC chickadees did not differ significantly from any other location, and the South Dakota BC chickadees had a significantly higher BMR than Ohio birds ($P = 0.009$).

Comparison of PMR within Species

Whole-organism and mass-corrected PMR did not differ between CA chickadees in Ohio and Tennessee (Fig. 5*B*). In contrast to CA chickadees, there was a significant effect of location on whole-organism PMR of BC chickadees ($F_{2,75} = 59.3$, $P < 0.001$); South Dakota birds had a significantly higher PMR than both Wisconsin and Ohio chickadees ($P < 0.001$ for both comparisons). Mass-corrected PMR of BC chickadees was also significantly different among populations ($F_{2,74} = 17.1$, $P < 0.001$), and again, South Dakota birds had a higher PMR than both
Wisconsin and Ohio birds ($P < 0.001$ for both comparisons). There was no significant difference between Wisconsin and Ohio BC chickadees in either whole-organism or mass-corrected PMR.

Metabolic Rate and Cold Tolerance across the Hybrid Zone

BC, CA, and hybrid chickadees from across the Ohio hybrid zone, and therefore from a similar environment, had significantly different BMRs (Fig. 6*A*; $F_{2,40} = 7.6$, $P = 0.002$). Carolina chickadees had a significantly lower BMR than hybrid ($P = 0.01$) and BC chickadees ($P = 0.002$), and there was no significant difference between BC and hybrid chickadees. There was also a significant difference in BMR among the Ohio BC,

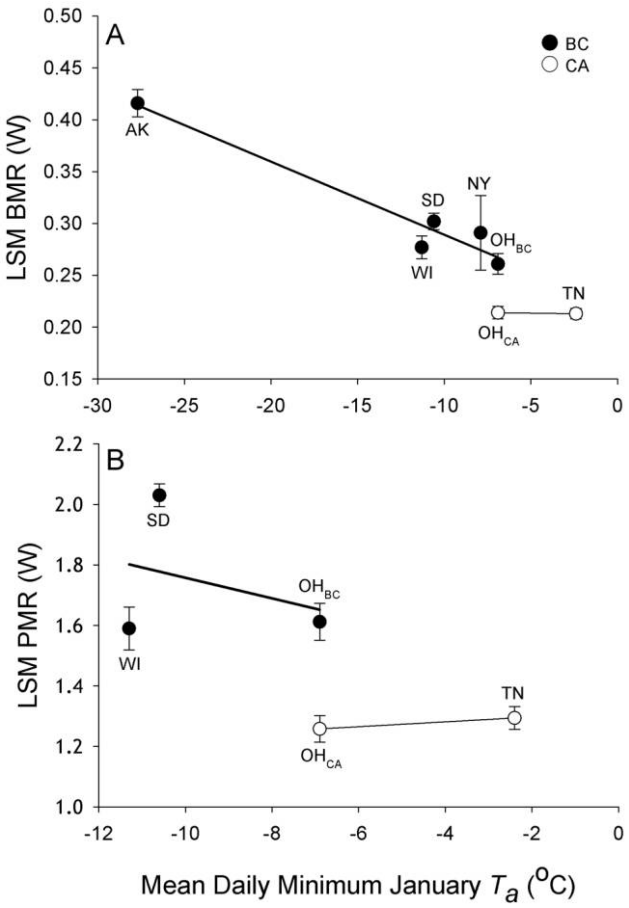

Figure 4. Mean basal metabolic rate (BMR; *A*) and peak metabolic rate (PMR; *B*) versus mean minimum January temperature for all locations included in this study. Metabolic rate is presented as least square means (LSM) controlling for mass. Linear regressions were calculated for both black-capped (BC; *solid circles*) and Carolina (CA; *open circles*) chickadees. AK = Alaska, WI = Wisconsin, SD = South Dakota, NY = New York, OH_{BC} = Ohio black-capped chickadee, OH_{CA} = Ohio Carolina chickadee, and TN = Tennessee.

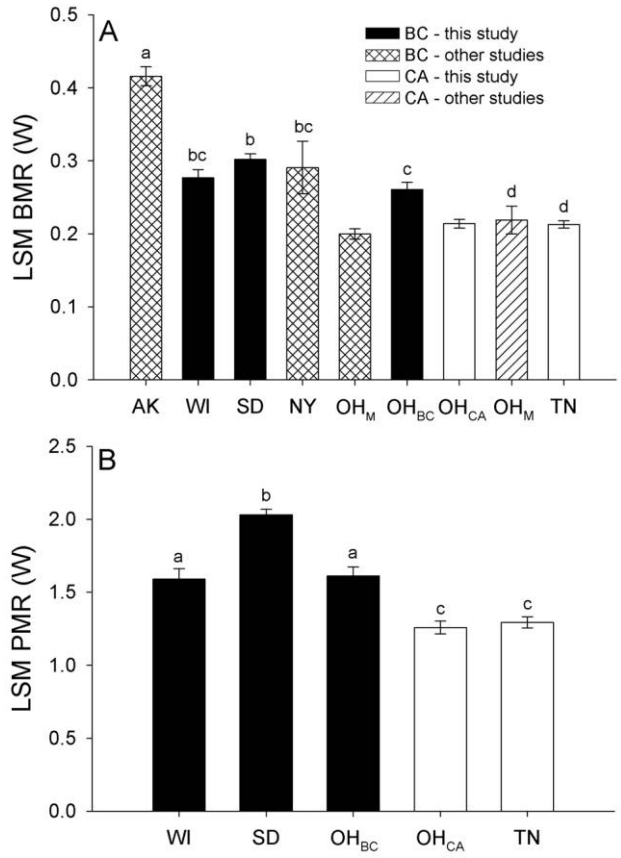

Figure 5. Mean basal metabolic rate (BMR; *A*) and peak metabolic rate (PMR; *B*) for Carolina (CA) and black-capped (BC) chickadees. Metabolic rate is presented as least square means (LSM) controlling for mass. Different letters identify significant differences in mass-correct metabolic rate between populations. Sample sizes can be found in Table 1. AK = Alaska, WI = Wisconsin, SD = South Dakota, NY = New York, OH_M = Ohio (Munzinger [1974] data), OH_{BC} = Ohio black-capped chickadee data from this study, OH_{CA} = Ohio Carolina chickadee data from this study, and TN = Tennessee.

CA, and hybrid chickadees after including mass as a covariate ($F_{2,39} = 6.1$, $P = 0.005$). Hybrids had a significantly higher mass-corrected BMR than either CA ($P = 0.01$) or BC ($P = 0.01$) chickadees, which did not differ significantly from each other.

PMR

Across the Ohio hybrid zone, we found that PMR was significantly related to genetic identity (Fig. 6*B*; $F_{2,31} = 6.0$, $P = 0.006$). PMR of CA chickadees was significantly lower than PMR of both BC ($P = 0.02$) and hybrid chickadees ($P = 0.02$), which did not differ significantly from each other. We did not find a significant difference in PMR between any of the three OH groups (CA, BC, hybrid) after controlling for mass.

Cold Tolerance

The average T_{cl} of BC chickadees, combining data for birds from Wisconsin and Ohio, did not differ significantly from the average T_{cl} of CA chickadees from Ohio and Tennessee (Fig. 7; $P = 0.07$). We did find a significant effect of location on T_{cl} within each species ($F_{3,48} = 6.9$, $P = 0.001$). The cold tolerance of Wisconsin chickadees was significantly higher than that of Ohio BC, Ohio CA, or Tennessee CA ($P < 0.01$ for all comparisons), which did not differ significantly from each other. There was no significant difference between the average T_{cl} of BC, CA, or hybrid chickadees across the Ohio hybrid zone.

Mitochondrial Haplotypes

For statistical analyses, we used AFLP to identify hybrids based on STRUCTURE scores. However, we did identify three BCs and one CA that possessed mtDNA of the other species, and these birds presumably represent some level of backcross hybrid. To ensure the accuracy of species identification in our analyses, we removed these four individuals and repeated all

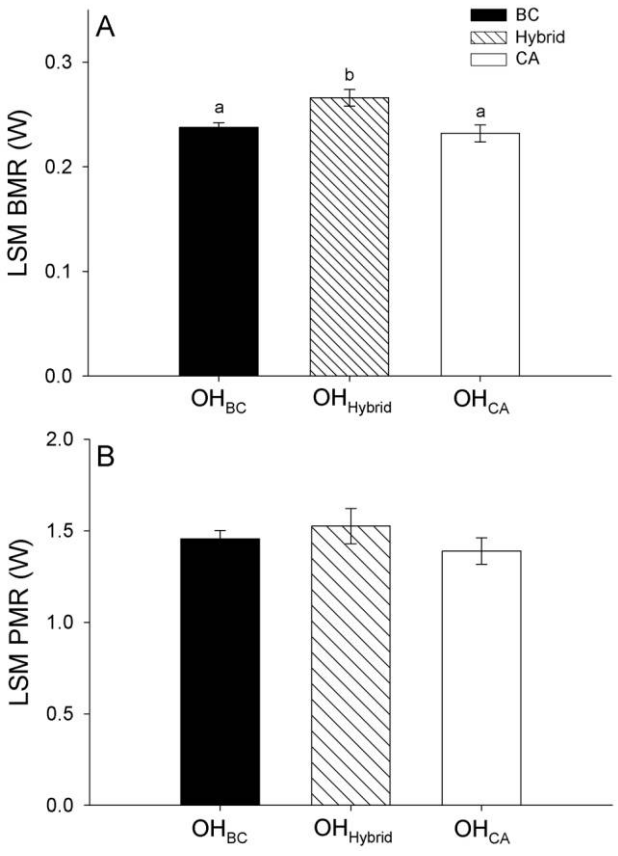

Figure 6. Mean basal metabolic rate (BMR; *A*) and peak metabolic rate (PMR; *B*) for each Ohio species. Metabolic rate is presented as least square means (LSM) controlling for mass, and different letters identify significant differences in mass-correct metabolic rate between populations. Sample sizes can be found in Table 1. OH_{BC} = Ohio black-capped chickadee, OH_{CA} = Ohio Carolina chickadee, and OH_{Hybrid} = Ohio hybrid chickadee.

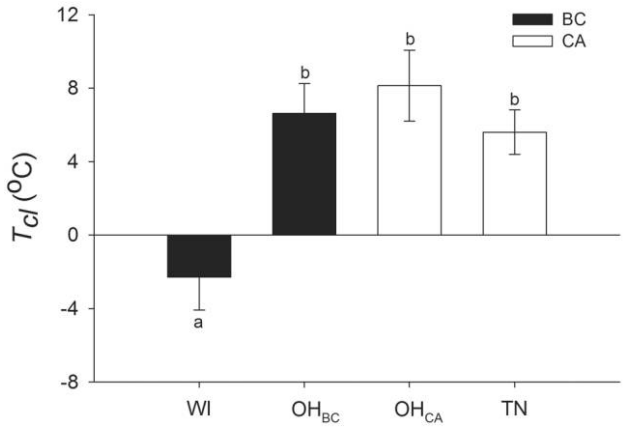

Figure 7. Mean T_{cl} for each chickadee population. Metabolic rate is presented as least square means controlling for mass, and different letters identify significant differences in mass-correct metabolic rate between populations. Sample sizes: WI $n = 11$, Ohio black-capped chickadee (OH_{BC}) $n = 18$, OH_{CA} = Ohio Carolina chickadee (OH_{CA}) $n = 10$, and TN $n = 13$.

statistical tests with the same results. Although these chickadees did not meet our a priori definition of a hybrid, we also repeated statistical tests with them classified as such. These tests rendered the comparison of BMR in Ohio birds insignificant, after including mass as a covariate ($F_{2,39} = 1.1$, $P = 0.3$). All other results remained the same.

Discussion

If chickadees maintain the minimum metabolic machinery necessary to survive ambient winter temperatures, their BMR and PMR should be associated with temperature isotherms. This "machinery-matching" strategy is consistent with the seasonal metabolic variation found in permanent resident, winter passerines (Hart 1962; Dawson and Marsh 1989; Marsh and Dawson 1989; O'Connor 1995; Likenes and Swanson 1996; Likenes 2005). In a previous study, winter BC chickadees had a standard metabolic rate that was 18% higher than that of summer birds (Cooper and Swanson 1994). In addition to seasonal variation, there is also evidence of significant winter-to-winter variation in both BMR and PMR of BC chickadees, which are negatively correlated with the mean minimum temperature of that season (Swanson and Olmstead 1999). This suggests that chickadees use seasonally variable, temperature-associated metabolic limits in order to facilitate winter survival. The presence of lower metabolic rates during both the summer and less severe winters implies some physiological cost to maintaining machinery that would result in increased metabolism year-round or during times when a lower level would suffice, although the physiological mechanisms controlling this variation remains unknown (Vézina et al. 2007; Swanson, forthcoming). Thus, we predicted that metabolic rates would be negatively correlated with temperature across our sampling sites: northern birds would have higher metabolic rates than conspecifics at lower latitudes.

Our data did support the notion that metabolism of BC chickadees is associated with winter temperatures (Fig. 4). We observed a significant correlation between lower temperatures and higher metabolic rates among all BC populations. Although there is a large range of temperatures that we did not sample between Alaska and Wisconsin, our conclusion is supported by the fact that this correlation remains significant even after removing Alaska birds from the analysis. However, this trend was not robust among all BC locations. For example, although Wisconsin was the coldest location sampled in the lower 48 states, the BMR of Wisconsin BC chickadees did not differ significantly from that of Ohio BC chickadees (the southernmost BC chickadee population), even before correcting for body mass. The mean minimum daily January temperatures of Wisconsin and Ohio differ by only 4.4°C, a difference that is less than the mean daily temperature span at either location (Wisconsin = 8.6°C, Ohio = 8.1°C; NOAA 2008). If chickadees in Ohio tolerate average daily temperature changes of 8.1°C, exposure to an environment that is, on average, only 4.4°C colder may not present a significant thermal challenge requiring a change in metabolic rate. However, other factors that we have not measured, such as wind and solar radiation, also influence heat flux in these small birds.

South Dakota BC chickadees had a significantly higher mass and PMR than any other BC chickadee population in the continental United States, even though the temperature profile of South Dakota is similar to that of Wisconsin. Researchers have consistently recorded masses around 13 g for this South Dakota population (Cooper and Swanson 1994; Dutenhoffer and Swanson 1996; Swanson and Likenes 2006). However, even after correcting for mass, these birds still had higher PMR than any other population in this study. Although possibly influenced by slight differences in methodology, such as rate of cooling during PMR tests (see Swanson 1990; Cooper and Swanson 1994), these data suggest that South Dakota chickadees have means other than increasing mass to elevate metabolic rate, such as elevating oxidative enzyme activity (Likenes 2005). It also suggests that this population may be exposed to environmental factors other than temperature that require an elevated metabolism. Southeastern South Dakota has the lowest forest cover, approximately 4% (Castonguay 1982), among all of our sampling sites, and the effects of both wind and temperatures below the thermoneutral zone become much more pronounced in isolated woodlots. Wind exposure can elevate metabolic rate in small birds (Bakken and Lee 1992; Dolby and Grubb 1999; Bakken et al. 2002). The exceedingly fragmented woodland landscape of South Dakota may amplify the effect of wind and explain in part why these birds had a higher metabolic rate than conspecifics at similar latitudes.

We saw little variation in mass-corrected BMR of chickadees across a wide range of latitudes (Fig. 5), suggesting that with the exception of Alaskan BC, chickadees may increase their body mass in response to lower T_a rather than altering tissue-specific rates of metabolism. Additional sampling locations may clarify whether Alaskan chickadees are simply an exception to this conclusion, or whether the trend observed in Figure 4 is robust throughout the BC range. Sharbaugh (2001) reported

measurements of Alaskan chickadee standard metabolic rates (SMR) that were closer to our measurements from the continental United States (SMR: 0.254 W in winter 1994, 0.338 W in winter 1995). However, the data for Alaskan birds must be interpreted with caution, as sampling protocols were not consistent with other locations: Alaskan chickadees in both of these studies (Grossman and West 1977; Sharbaugh 2001) were housed in outdoor cages with ad lib. food throughout the winter. In addition, Grossman and West (1977) used only five birds for multiple measurements, indicating that at least three of the data points used in our analysis are cases of pseudoreplication.

The Ohio chickadees represent a moving hybrid zone with forces opposing gene flow between the two species. Most hybrid zones are maintained by some form of endogenous selection (Barton and Gale 1981, 1985, 1989), which favors alleles within a complementary genetic background and selects against individuals of mixed ancestry (Barton and Gale 1993). Despite evidence of gene flow across the contact zone (Sattler and Braun 2000), previous studies revealed that there is selection against hybrid reproductive success (Bronson et al. 2003*a*). These results suggested to us that the genetic incompatibilities of hybrids might have additional physiological manifestations beyond reproduction.

Ohio chickadees provided us with the opportunity to explore the metabolic consequences of being a hybrid. One hypothesis is that there will be no observable difference between hybrids and parental populations, either because selection has already removed individuals with severe mitochondrial inefficiency during the months preceding our sampling or because the two species' genomes are so similar that metabolism is not penalized by hybridization. However, we predicted that Ohio hybrids would have a higher BMR and a lower PMR than BC or CA chickadees in the same environment. Due to the role of mitochondrial function as a regulator of metabolism (Tieleman et al. 2009), a mismatch of proteins encoded by nuclear and mitochondrial genes, such as could be the case in hybrids, may not produce a properly functioning metabolic system due to misassembled metabolic proteins. In our study, Ohio hybrids did have a higher BMR than both BC and CA chickadees, after including mass as a covariate. Although speculative, we think that the higher BMR of hybrid chickadees may be due to genetically induced mitochondrial deficiencies.

Several recent studies have suggested that nDNA-mtDNA interactions are disrupted by hybridization events, impairing the function of metabolic enzymes—specifically those encoded in the mtDNA (Breeuwer and Werren 1995; Edmands and Burton 1999; Sackton et al. 2003; Perrot-Minnot et al. 2004; Zeyl et al. 2005; Ellison and Burton 2008). For example, cytochrome c oxidase (COX; complex IV of the electron transport system) contains 13 subunits, three of which are mitochondrial encoded (Grossman et al. 2004). In addition to the reduced fecundity and viability observed in interpopulation hybrid marine copepods (*Tigriopus californicus*; Edmands 1999), Edmands and Burton (1999) also detected reduced COX activity in individuals with mtDNA subunits from one population and a mixed (hybrid) nuclear background. Further, the low fitness and reduced ATP synthesis of F_3 hybrids can be restored through maternal but not paternal backcrosses, which allow the parental nuclear-mitochondrial genomes to be reassembled. Although BC and CA chickadees have a relatively small divergence in nuclear DNA (<0.3%, based on restriction fragment mapping), they exhibit a mitochondrial DNA sequence divergence of about 7% (Mack et al. 1986; Sawaya 1990).

Ohio BC chickadees were larger, and they did not differ from hybrid or CA chickadees with respect to PMR or cold tolerance after including mass as a covariate. PMR reflects the maximum limit of oxidative respiration in muscle cells, and for many winter passerines, heat production facilitated by shivering thermogenesis is energetically demanding. We proposed that Ohio could be the northern thermal limit of the CA chickadee population because of the relationship between temperature isotherms and the hybrid zone. Thus, we predicted that higher PMRs would convey an advantage to Ohio BC chickadees over Ohio CA chickadees during periods of thermal challenge. It does appear that BC chickadees are better adapted to the cold weather found in northern areas of Ohio, and the northward movement of CA chickadees may be facilitated by the increase in winter temperatures observed over the past half-century.

Acknowledgments

We thank the landowners in Ohio and Tennessee, especially Steve Humrichauser and Judy Varner, who allowed us to capture birds on their properties. We would also like to acknowledge Sue Chaplin for going beyond the call of duty to find New York chickadee data that were covered in dust; Chris Huddleston and Sarah Kingston for their assistance and advice in our genetic identification of chickadees; the Williams Lab group for their comments on earlier drafts of this manuscript; Thomas Grubb for his role in initially suggesting this project; and the comments of three anonymous reviewers. This study was supported in part by National Science Foundation IBN-0212587 to J.B.W.

Literature Cited

Aschoff J. and H. Pohl. 1970. Rhythmic variations in energy metabolism. Fed Proc 29:1541–1552.

Bakken G.S. and K.F. Lee. 1992. Effects of wind and illumination on behavior and metabolic rate of American goldfinches (*Carduelis tristis*). Auk 109:119–125.

Bakken G.S., J.B. Williams, and R.E. Ricklefs. 2002. Metabolic response to wind of downy chicks of arctic-breeding shorebirds (Scolopacidae). J Exp Biol 205:3435–3443.

Bartholomew G.A., D. Vleck, and C.M. Vleck. 1981. Instantaneous measurements of oxygen consumption during preflight warm-up and post-flight cooling in sphingid and saturniid moths. J Exp Biol 90:17–32.

Barton N.H. and K.S. Gale. 1981. Hybrid zones and speciation. Pp. 109–145 in W.R. Atchley and D.S. Woodruff, eds. Evo-

lution and Speciation: Essays in Honor of M. J. D. White. Cambridge University Press, Cambridge.
———. 1985. Analysis of hybrid zones. Annu Rev Ecol Syst 16:113–148.
———. 1989. Adaptation, speciation and hybrid zones. Nature 341:497–503.
———. 1993. Genetic analysis of hybrid zones. Pages 13–45 in R. Harrison, ed. Hybrid Zones and the Evolutionary Process. Oxford University Press, New York.
Bennett A.F. 1991. The evolution of activity capacity. J Exp Biol 160:1–23.
Breeuwer J.A.J. and J.H. Werren. 1995. Hybrid breakdown between two haplodiploid species—the roles of nuclear and cytoplasmic genes. Evolution 49:705–717.
Brewer R. 1963. Ecological and reproductive relationships of black-capped and Carolina chickadees. Auk 80:9–47.
Bronson C.L., T.C. Grubb, and M.J. Braun. 2003*a*. A test of the endogenous and exogenous selection hypotheses for the maintenance of a narrow avian hybrid zone. Evolution 57: 630–637.
Bronson C.L., T.C. Grubb, G.D. Sattler, and M.J. Braun. 2003*b*. Mate preference: a possible causal mechanism for a moving hybrid zone. Anim Behav 65:489–500.
———. 2005. Reproductive success across the black-capped chickadee (*Poecile atricapillus*) and Carolina chickadee (*P. carolinensis*) hybrid zone in Ohio. Auk 122:759–772.
Bryant D.M. 1997. Energy expenditure in wild birds. Proc Nutr Soc 56:1025–1039.
Canada Department of Energy, Mines and Resources. 1974. The National Atlas of Canada. Macmillan, Toronto.
Canterbury G. 2002. Metabolic adaptation and climatic constraints on winter bird distribution. Ecology 83:946–957.
Castonguay M. 1982. Forest Area in Eastern South Dakota, 1980. Research Note NC-291. North Central Forest Experiment Station, St. Paul, MN.
Castro G. 1989. Energy costs and avian distributions: limitations or chance? a comment. Ecology 70:1181–1182.
Chaplin S.B. 1974. Daily energetics of black-capped chickadee, *Parus atricapillus*, in winter. J Comp Physiol 89:321–330
Cooper S.J. and D.L. Swanson. 1994. Seasonal acclimatization of thermoregulation in the black-capped chickadee. Condor 96:638–646.
Daan S., B.M. Barnes, and A.M. Strijkstra. 1991. Warming up for sleep? ground squirrels sleep during arousals from hibernation. Neurosci Lett 128:265–268.
Dawson W.R. and R.L. Marsh. 1989. Metabolic acclimatization to cold and season in birds. Pp. 83–94 in C. Bech and R.E. Reinertsen, eds. Physiology of Cold Adaptation in Birds. Plenum, New York.
Dobzhansky T. 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics 21:113–135.
Dolby A.S. and T.C. Grubb. 1999. Effects of winter weather on horizontal vertical use of isolated forest fragments by bark-foraging birds. Condor 101:408–412.
Dutenhoffer M.S. and D.L. Swanson. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiol Zool 69:1232–1254.
Edmands S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53:1757–1765
Edmands S. and R.S. Burton. 1999. Cytochrome c oxidase activity in interpopulation hybrids of a marine copepod: a test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. Evolution 53:1972–1978.
Ellison C.K. and R.S. Burton. 2008. Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. Proc Natl Acad Sci USA 105:15831–15836.
Grossman A.F. and G.C. West. 1977. Metabolic rate and temperature regulation of winter acclimatized black-capped chickadees *Parus atricapillus* of interior Alaska. Ornis Scand 8:127–138.
Grossman L.I., D.E. Wildman, T.R. Schmidt, and M. Goodman. 2004. Accelerated evolution of the electron transport chain in anthropoid primates. Trends Genet 20:578–585.
Grubb T.C., R.A. Mauck, and S.L. Earnst. 1994. On no-chickadee zones in midwestern North America: evidence from the Ohio Breeding Bird Atlas and the North American Breeding Bird Survey. Auk 111:191–197.
Harper M.E., R. Dent, S. Monemdjou, V. Bezaire, L. Van Wyck, G. Wells, G.N. Kavaslar, A. Gauthier, F. Tesson, and R. McPherson. 2002. Decreased mitochondrial proton leak and reduced expression of uncoupling protein 3 in skeletal muscle of obese diet-resistant women. Diabetes 51:2459–2466.
Hart J. 1962. Seasonal acclimatization in four species of small wild birds. Physiol Zool 35:224–236.
Hayes J.P. 2001. Mass-specific and whole-animal metabolism are not the same concept. Physiol Biochem Zool 74:147–150.
Hill R.W. 1972. Determination of oxygen consumption by use of paramagnetic oxygen analyzer. J Appl Physiol 33:261–263.
Hinds D.S. and C.N. Rice-Warner. 1992. Maximum metabolism and aerobic capacity in heteromyid and other rodents. Physiol Zool 65:188–214.
Hoppeler H. and E.R. Weibel. 1998. Limits for oxygen and substrate transport in mammals. J Exp Biol 201:1051–1064.
King J. and D. Farner. 1961. Energy metabolism, thermoregulation and body temperature. Pp. 215–288 in A. Marshall, ed. Biology and Comparative Physiology of Birds. Academic Press, New York.
Kingston S.E. and P.E. Rosel. 2004. Genetic differentiation among recently diverged delphinid taxa determined using AFLP markers. J Hered 95:1–10.
Koteja P. 1987. On the relation between basal and maximum metabolic rate in mammals. Comp Biochem Physiol A 87: 205–208.
Lasiewski R. and W. Dawson. 1967. A re-examination of relation between standard metabolic rate and body weight in birds. Condor 69:13–23.
Levy A. 1964. Accuracy of bubble meter method for gas flow measurements. J Sci Meas 41:449.

Likenes E.T. 2005. Seasonal Acclimatization Patterns and Mechanisms in Small, Temperate-Resident Passerines: Phenotypic Flexibility of Complex Traits. PhD diss. University of South Dakota.

Likenes E.T. and D.L. Swanson. 1996. Seasonal variation in cold tolerance, basal metabolic rate, and maximal capacity for thermogenesis in white-breasted nuthatches, *Sitta carolinensis*, and downy woodpeckers, *Picoides pubescens*, two unrelated arboreal temperate residents. J Avian Biol 27:279–288.

Longmire J., A. Lewis, N. Brown, J. Buckingham, L. Clark, M. Jones, L.J. Meincke, et al. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2:14–24.

Mack A.L., F.B. Gill, R. Colburn, and C. Spolsky. 1986. Mitochondrial DNA: a source of genetic markers for studies of similar passerine bird species. Auk 103:676–681.

Marsh R.L. and W.R. Dawson. 1989. Avian adjustments to cold. Pp. 206–253 in C.H. Wang, ed. Animal Adaptation to Cold. Vol. 4 of Advances in Comparative and Environmental Physiology. Springer, Berlin.

McNab B.K. 1997. On the utility of uniformity in the definition of basal rate of metabolism. Physiol Zool 70:718–720.

Munzinger J. 1974. A Comparative Study on the Energetics of the Black-Capped and Carolina Chickadees, *Parus atricapillus* and *Parus carolinensis*. PhD diss. Ohio State University.

NOAA (National Oceanic and Atmospheric Administration). 2008. Record of climatological observations. National Climatic Data Center, Asheville, NC. http://cdo.ncdc.noaa.gov/dly/DLY.

O'Connor T.P. 1995. Metabolic characteristics and body composition in house finches: effects of seasonal acclimatization. J Comp Physiol B 165:298–305.

Packard G.C. and T.J. Boardman. 1988. The misuse of ratios, indexes, and percentages in ecophysiological research. Physiol Zool 61:1–9.

———. 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? Comp Biochem Physiol A 122: 37–44.

Perrot-Minnot M.-J., A. Migeon, and M. Navajas. 2004. Intergenomic interations affect female reproduction: evidence from introgression and inbreeding depression in a haplodiploid mite. Heredity 93:551–558

Platt J. 1964. Strong inference. Science 146:347–353.

Pon L.A. and E.A. Schon. 2001. Mitochondria. Academic Press, St. Louis.

Porter W.P. 1969. Thermal radiation in metabolic chambers. Science 166:115–117.

Pritchard J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959

Repasky R.R. 1991. Temperature and the northern distributions of wintering birds. Ecology 72:2274–2285.

Reudink M.W., S.G. Mech, and R.L. Curry. 2006. Extrapair paternity and mate choice in a chickadee hybrid zone. Behav Ecol 17:56–62.

Reudink M.W., S.G. Mech, S.P. Mullen, and R.L. Curry. 2007. Structure and dynamics of the hybrid zone between black-capped chickadee (*Poecile atricapillus*) and Carolina chickadee (*P. carolinensis*) in southeastern Pennsylvania. Auk 124: 463–478.

Rezende E.L., D.L. Swanson, F.F. Novoa, and F. Bozinovic. 2002. Passerines versus nonpasserines: so far, no statistical differences in scaling of avian energetics. J Exp Biol 205:101–107.

Rising J.D. 1968. A multivariate assessment of interbreeding between the chickadees *Parus atricapillus* and *P. carolinensis*. Syst Zool 17:160–169.

Rising J.D. and J.W. Hudson. 1974. Seasonal variation in the metabolism and thyroid activity of the black-capped chickadee (*Parus atricapillus*). Condor 76:298–303.

Robbins, M.B., M.J. Braun, and E.A. Tobey. 1986. Morphological and vocal variation across a contact zone between the chickadees *Parus atricapillus* and *Parus carolinensis*. Auk 103: 655–666.

Rolfe D.F.S. and G.C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 77:731–758.

Root T. 1988*a*. Energy constraints on avian distributions and abundances. Ecology 69:330–339.

———. 1988*b*. Environmental factors associated with avian distributional boundaries. J Biogeogr 15:489–505.

———. 1989. Energy constraints on avian distributions: a reply to Castro. Ecology 70:1183–1185.

Rosenmann M. and P. Morrison. 1974. Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-O_2. Am J Physiol 226:490–495.

Saarela S., B. Klapper, and G. Heldmaier. 1995. Daily rhythm of oxygen consumption and thermoregulatory responses in some European winter- or summer-acclimatized finches at different ambient temperatures. J Comp Physiol B 165:366–376.

Sackton T.B., R.A. Haney, and D.M. Rand. 2003. Cytonuclear coadaptation in *Drosophila*: disruption of cytochrome c oxidase activity in backcross genotypes. Evolution 57:2315–2325.

Sattler G.D. and M.J. Braun. 2000. Morphometric variation as an indicator of genetic interactions between black-capped and Carolina chickadees at a contact zone in the Appalachian mountains. Auk 117:427–444.

Sawaya P. 1990. A Detailed Analysis of the Genetic Interaction at a Hybrid Zone between the Chickadees *Parus atricapillus* and *P. carolinensis* as Revealed by Nuclear and Mitochondrial DNA Restriction Fragment Length Variation. PhD diss. University of Cincinnati.

Sharbaugh S.M. 2001. Seasonal acclimatization to extreme climatic conditions by black-capped chickadees (*Poecile atricapilla*) in interior Alaska (64°N). Physiol Biochem Zool 74: 568–575.

Smith S.M. 1993. Black-capped chickadee (*Poecile atricapillus*), in A. Poole, ed. The Birds of North America Online. Cornell Lab of Ornithology, Ithaca, NY. http://bna.birds.cornell.edu/bna/species/039.

Stuart J.A., K.M. Brindle, J.A. Harper, and M.D. Brand. 1999. Mitochondrial proton leak and the uncoupling proteins. J Bioenerg Biomembr 31:517–525.

Swanson D. 1990. Seasonal variation in cold hardiness and peak rates of cold induced thermogenesis in the dark-eyed junco (*Junco hyemalis*). Auk 107:561–566.

———. 2001. Are summit metabolism and thermogenic endurance correlated in winter-acclimatized passerine birds? J Comp Physiol B 171:475–481.

———. Forthcoming. Seasonal metabolic variation in birds: functional and mechanistic correlates. Curr Ornithol.

Swanson D.L., M.W. Drymalski, and J.R. Brown. 1996. Sliding vs. static cold exposure and the measurement of summit metabolism in birds. J Therm Biol 21:221–226.

Swanson D.L. and E.T. Likenes. 2006. A comparative analysis of thermogenic capacity and cold tolerance in small birds. J Exp Biol 209:466–474.

Swanson D.L. and K.L. Olmstead. 1999. Evidence for a proximate influence of winter temperature on metabolism in passerine birds. Physiol Biochem Zool 72:566–575.

Taigen T.L. 1983. Activity metabolism of anuran amphibians: implications for the origin of endothermy. Am Nat 121:94–109.

Tanner J.T. 1952. Black-capped and Carolina chickadees in the southern Appalachian Mountains. Auk 69:407–442.

Tieleman B., M. Versteegh, A. Fries, B. Helm, H. Gibbs, and J.B. Williams. 2009. Genetic modulation of energy metabolism in birds through mitochondrial function. Proc R Soc B 276:1685–1693.

Trautman M. 1940. The Birds of Buckeye Lake, Ohio. Univ Mich Mus Zool Misc Pub 44.

U.S. Geological Survey. 1970. The National Atlas of the United States of America. U.S. Department of the Interior, Washington, DC.

Vézina F., K.M. Jalvingh, A. Dekinga, and T. Piersma. 2006. Acclimation to different thermal conditions in a northerly wintering shorebird is driven by body mass-related changes in organ size. J Exp Biol 209:3141–3154.

———. 2007. Thermogenic side effects to migratory predisposition in shorebirds. Am J Physiol 292:R1287–R1297.

Vos P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414.

Weathers W.W. 1979. Climatic adaptation in avian standard metabolic rate. Oecologia 42:81–89.

Weibel E.R., L.D. Bacigalupe, B. Schmitt, and H. Hoppeler. 2004. Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. Respir Physiol Neurobiol 140:115–132.

Weibel E.R. and H. Hoppeler. 2005. Exercise-induced maximal metabolic rate scales with muscle aerobic capacity. J Exp Biol 208:1635–1644.

Wheaton J. 1882. Report on the birds of Ohio. Ohio Geol Surv Bull 4:187–623.

White C.R., P. Cassey, and T.M. Blackburn. 2007. Allometric experiments do not support a universal metabolic allometry. Ecology 88:315–323.

White C.R. and R.S. Seymour. 2004. Does basal metabolic rate contain a useful signal? mammalian BMR allometry and correlations with a selection of physiological, ecological, and life-history variables. Physiol Biochem Zool 77:929–941.

Wiersma P., M.A. Chappell, and J.B. Williams. 2007*a*. Cold- and exercise-induced peak metabolic rates in tropical birds. Proc Natl Acad Sci USA 104:20866–20871.

Wiersma P., A. Munoz-Garcia, A. Walker, and J.B. Williams. 2007*b*. Tropical birds have a slow pace of life. Proc Natl Acad Sci USA 104:9340–9345.

Zeyl C., B. Andreson, and E. Weninck. 2005. Nuclear-mitochondrial epistasis for fitness in *Saccharomyces cerevisiae*. Evolution 59:910–914.