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

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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.

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. 2007b). 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. 2007a). 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. 2007a), 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 1988b, 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 (1988b) found
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 1988a; 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-

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 nonmutually 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. (2003a). 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. (2003a). 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-
measurements 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 Tc 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 CO2, 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, Vo2, 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). Incur- rent 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 Tc 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 (Tcl; Saarela et al. 1995), which was the temperature at which a bird achieved PMR during cold trials, and used Tc 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°C 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 Board- man 1988, 1999; Hayes 2001). Data presented in figures are least square means ± 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. 2003a). 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. (2003a) 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.

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_{1,49} = 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 BMR (W) = −1.42 + 0.77 log mass (g) (R² = 0.22, P = 0.02), whereas for BC chickadees, it was log BMR (W) = −1.98 + 1.35 log mass (g) (R² = 0.45, P < 0.001). Slopes and intercepts of equations for BMR did not differ significantly between species.

For CA chickadees, log PMR (W) = −1.19 + 1.34 log mass (g) (R² = 0.33, P = 0.004), and for BC chickadees, log PMR (W) = −0.99 + 1.12 log mass (g) (R² = 0.52, P < 0.001). Slopes and intercepts of PMR equations did not differ significantly between species.

Tₚ 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: R² = 0.61, P < 0.001; PMR: R² = 0.42, P < 0.001). The relationships between metabolic rate and temperature are as follows: BMR (W) = 0.191 − 0.009 Tₚ (°C); PMR (W) = 0.942 − 0.092 Tₚ (°C). When examining only populations of black-capped chickadees, this relationship remained significant: BMR (W) = 0.191 − 0.009 Tₚ (°C), R² = 0.54, P < 0.001; PMR (W) = 0.821 − 0.107 Tₚ (°C), R² = 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 Tₚ (°C), R² = 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_{1,98} = 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. 5A). 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_{3,76} = 40.8, P < 0.001), although more northerly populations did not consistently have higher BMRs. Alaskan BC had a significantly higher BMR than
<table>
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<th>Latitude</th>
<th>$T_{\text{min}}$ (°C)$^a$</th>
<th>Mass (g)$^b$</th>
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<th>PMR (W)</th>
<th>$n_{\text{PMR}}$</th>
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Note. BMR = basal metabolic rate; PMR = peak metabolic rate; $T_{cl}$ = temperature at the cold limit.


*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.

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, OHBC = Ohio black-capped chickadee, OHCA = Ohio Carolina chickadee, and TN = Tennessee.

Comparison of PMR within Species

Whole-organism and mass-corrected PMR did not differ between CA chickadees in Ohio and Tennessee (Fig. 5B). In contrast to CA chickadees, there was a significant effect of location on whole-organism PMR of BC chickadees (F_{2,76} = 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. 6A; F_{2,40} = 7.6, P = 0.0022). 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,
Across the Ohio hybrid zone, we found that PMR was significantly related to genetic identity (Fig. 6B; $F_{2,31} = 6.0$, $P = 0.006$). 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 ($F_{3,43} = 6.9$, $P = 0.001$). 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
metabolic rates at lower latitudes. Thus, we predicted that metabolic rates would be negatively correlated with temperature isotherms. This implies some physiological cost to maintaining machinery that is 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 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.

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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. 2003a). 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 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 Humrichauer 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 Hudleston 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 ISBN-0212587 to J.B.W.

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