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Relationships between interspecific differences in the mass of internal organs, biochemical markers of metabolic activity, and the thermogenic properties of three small passerines

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Abstract

Background: The capacity for thermogenesis is considered part of an animal's adaptive strategy for survival, and basal metabolic rate (BMR) is one of the fundamental physiological standards for assessing the energy cost of thermoregulation in endotherms. BMR has been shown to be a highly flexible phenotypic trait both between, and within, species, but the metabolic mechanisms involved in the regulation of BMR, which range from variation in organ mass to biochemical adjustments, remain unclear. In this study, we investigated the relationship between organ mass, biochemical markers of metabolic tissue activity, and thermogenesis, in three species of small passerines: wild Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*), caught in Wenzhou, southeastern China.

Methods: Oxygen consumption was measured using an open-circuit respirometry system. Mitochondrial state-4 respiration and cytochrome c oxidase (COX) activity in liver and pectoral muscle were measured with a Clark electrode.

Results: Our results show that Eurasian Tree Sparrows had significantly higher BMR, digestive organ mass, mitochondrial state-4 respiration capacity and COX activity in liver and muscle, than Bramblings and Little Buntings. Furthermore, interspecific differences in BMR were strongly correlated with those indigestive tract mass, state-4 respiration and COX activity.

Conclusions: Our findings suggest that the digestive organ mass, state-4 respiration and COX activity play an important role in determining interspecific differences in BMR.

Keywords: Basal metabolic rate (BMR), Cytochrome c oxidase (COX), State-4 respiration, *Fringilla montifringilla*, *Emberiza pusilla*, *Passer montanus*

Background

Adaptive variation in thermogenic capacity is critical to the survival of small birds in temperate zones (Schmidt-Nielsen 1997; Weathers 1997). To minimize the energetic cost of thermoregulation, birds use a variety of morphological and behavioral traits to adjust their rates of heat loss and heat gain, ranging from biochemical adjustments to changes in internal and whole organ mass (McKechnie 2008; Swanson 2010; Zheng et al. 2014a). Basal metabolic rate (BMR) refers to the energy expenditure of an animal at rest (i.e. thermoneutrality) during the inactive phase of the day, when it is not processing food, moulting, or reproducing (McNab 2009), and is one of the fundamental physiological standards for assessing the energetic cost of thermoregulation (McKechnie

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et al. 2006). BMR has been referred to as a fundamental energetic trait, in large part because it represents a fixed cost that all organisms must incur (Furness 2003). BMR can consume as much as 50–60 % of daily energy expenditure (DEE) and variation in BMR may be associated with peak, or sustained, metabolic rates, species richness and distribution, activity levels and life-history strategies (McKinney and McWilliams 2005; Wells and Schaeffer 2012). BMR is therefore an important parameter for both inter- and intraspecific comparisons of thermoregulatory ability (McKechnie et al. 2006; McNab 2009). To date, BMR has been measured in more than 500 species of birds (McKechnie 2008; McNab 2009; Smit and McKechnie 2010). Those studies show that BMR is highly flexible both between and within species (Wikelski et al. 2003; Wiersma et al. 2007; Zheng et al. 2014a). The typically lower BMR of tropical birds compared to that of their high-latitude counterparts has been explained as an adaptation to avoid heat stress and to conserve water (Wiersma et al. 2007). In turn, the higher BMR of temperate birds has been explained as a direct or indirect result of adaptation to a colder climate and a shorter breeding season, both of which would be expected to require a higher level of metabolic activity (Swanson 2010; Zheng et al. 2014b). It has been suggested that the BMR of birds that migrate to tropical latitudes in winter, but breed in colder, temperate latitudes in summer, is lower in winter than in summer (Lindström and Klaassen 2003; Zheng et al. 2013a).

What are the metabolic mechanisms underlying variation in BMR? McKechnie (2008) and Swanson (2010) identified major physiological and morphological pathways whereby metabolic rates are up- or down-regulated, namely, adjustments in organ mass, adjustments in the mass-specific metabolic intensities of specific organs, and adjustments in the transport capacities for oxygen and metabolic substrates (Zheng et al. 2014b). At the organism level, the mechanism that has received the most attention has been the positive relationship between body mass (M_b) adjusted organ size and BMR (Daan et al. 1990; Chappell et al. 1999; Hammond et al. 2000). Although they represent less than 10 % of M_b, internal organs such as the liver, kidneys, heart and digestive tract can contribute more than 60 % of the energy expended at the basal level (Rolfe and Brown 1997; Clapham 2012). The liver is one of the largest and most metabolically active organs in endotherms, and, under basal metabolic conditions, may contribute 25 % of total heat production (Villarin et al. 2003; Zheng et al. 2008a). Skeletal muscles have lower, mass-specific, resting metabolic rates than many central organs (Scott and Evans 1992). However, due to their large total mass, they may contribute significantly to seasonal metabolic acclimatization (Chappell et al. 1999; Zheng et al. 2008b, 2014a). At the physiological and biochemical level, changes in activities of catabolic enzymes could influence the mass-specific metabolic intensities of organs, thereby affecting BMR (Liknes and Swanson 2011; Zheng et al. 2014a). Such variation in cellular metabolic intensity is often measured by examining variation in state-4 respiration (Zheng et al. 2008b, 2013a), citrate synthase (CS) activity (Swanson 2010; Swanson et al. 2014), or cytochrome coxidase (COX) activity. CS plays a key role in the Krebs cycle whereas state-4 respiration and COX are important in oxidative phosphorylation (Zheng et al. 2014a, b). The adaptive changes that produce higher BMR in small birds are thought to have a cellular or molecular basis and levels of state-4 respiration and COX activity have been commonly used as enzymatic indicators of variation in BMR at the cellular level (Zheng et al. 2008b, 2014a; Zhou et al. 2016).

Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*) inhabit vast areas of Europe and Asia (MacKinnon and Phillipps 2000). Bramblings and Little Buntings are migratory, wintering in southern Europe, northern India, and China, whereas Eurasian Tree Sparrows are resident in China. Bramblings and Little Buntings migrate to Wenzhou only during spring and autumn migration periods (Liu et al. 2001; Zheng et al. 2013a). The Brambling and Little Bunting have relatively higher body temperatures and metabolic rates than expected based on their body masses and broad thermal neutral zones, and relatively lower critical temperatures (Liu et al. 2001, 2004). The Eurasian Tree Sparrow increases its thermogenic capacity in cold conditions mainly by increasing both respiratory enzyme activity and the level of plasma thyroid hormones (Liu et al. 2008; Zheng et al. 2008a, 2014b). The capacity to make these metabolic adjustments may be the key for this species being able to survive in relatively cold areas (Liu et al. 2004, 2008). The present study is a continuation of investigation into the thermogenic capacities of these species. We hypothesized that species-specific physiological and biochemical metabolic characteristics would contribute to interspecific variation in BMR. We predicted that species with relatively high BMR would have higher organ mass, mitochondrial respiration capacity and COX activity. In this study we tested this hypothesis by comparing BMR, organ mass and selected biochemical markers of metabolic cellular activity, in these three species.

Methods

Animals

Seven Bramblings and eight Little Buntings were live-trapped in forested parts of Wenzhou, Zhejiang Province (27°29′N, 120°51′E) in China during the spring migration period in 2011, and ten Eurasian Tree Sparrows were captured at the same time. The Wenzhou climate is warm-temperate with an average annual rainfall of 1700 mm spread across all months with slightly more precipitation during winter and spring. Mean daily maximum temperatures range from 39 °C in July to 8 °C in January (Zheng et al. 2008a, 2014a). Body mass (M_b) to the nearest 0.1 g was determined immediately upon capture with a Sartorius balance (model BT25S). Bramblings, buntings and sparrows were transported to the laboratory and caged for 1 or 2 d (50 cm × 30 cm × 20 cm) outdoors under natural photoperiod (about 14:10 hours light:dark photoperiod) and temperature (18 °C) before measurements. Food and water were supplied *ad libitum* (Zhou et al. 2016). All experimental procedures were approved by the Animal Care and Use Committee of the Wenzhou City, Zhejiang Province, China (Wu et al. 2015; Zhou et al. 2016).

Measurement of metabolic rate

We measured oxygen consumption using an open-circuit respirometry system (S-3A/I, AEI technologies, Pittsburgh, PA, USA) (Zheng et al. 2014a). We provided a perch in respirometry chamber and allowed individual birds to rest in the 1.5-L metabolic chamber before measuring their metabolic rate (Smit and McKechnie 2010). The metabolic chamber was housed in a temperature-controlled cabinet capable of regulating temperature to ±0.5 °C (Artificial Climatic Engine BIC-300, Shanghai, China). H_2O and CO_2 were scrubbed from the air with a silica gel/soda lime/silica column before and after it passed through the metabolic chamber. We determined the fractional concentrations of oxygen in the inlet and outlet chamber air with an oxygen sensor (AEI technologies N-22M, USA). During the measurement of metabolic rates, we pumped dry CO_2-free air through the chamber at 300 mL/min with a flow control system (AEI technologies R-1, USA) calibrated to ±1 % accuracy with a general purpose thermal mass flow-meter (TSI 4100 Series, USA), to maintain the fractional concentration of O_2 in the chamber at about 20 % (McNab 2006). We obtained the baseline O_2 concentration before and after each test (Li et al. 2010; Wu et al. 2015). We measured oxygen consumption rates at 30 ± 0.5 °C, which is within the thermal neutral zone for Bramblings, Little Buntings and Eurasian Tree Sparrows (Zheng et al. 2008b, 2014b). We obtained all measurements of gas exchange during the rest-phase of birds' circadian cycles (between 20:00 and 04:00 hours) in dark chambers. We removed food 4 h before each test to create post-absorptive conditions. Measurement of oxygen consumption commenced when birds were observed perching calmly in the chamber and continued for 1 h. In general, each animal was in the metabolic chamber for at least 2 h. The oxygen consumption data were recorded every minute according to the equation 2 described by Hill (1972). We took the lowest 5 min mean oxygen consumption data over the test period to calculate BMR (Wu et al. 2015; Zhou et al. 2016). We expressed metabolic rates as mL O_2/h after correcting all values to standard temperature, pressure, and dry gas (STPD) conditions (Schmidt-Nielsen 1997). We measured body temperature during metabolic measurements using a lubricated thermocouple inserted in the cloaca, and digitized the output using a thermocouple meter (Beijing Normal University Instruments Co.). We measured M_b to the nearest 0.1 g before and after the experiments, and used mean M_b in calculations. All measurements were taken daily between 20:00 and 04:00 hours.

Measurement of organ mass

Birds were euthanized by cervical dislocation at the end of the experiment and their pectoral muscle, heart, liver, kidneys, gizzard, small intestine and rectum extracted and weighed to the nearest 0.1 mg. Part of the muscle and liver was used to investigate state-4 respiration and COX activity (Zheng et al. 2008b, 2014a), and the other internal organs, including the remainder of the muscle and liver, were dried to a constant mass over 2 days at 65 °C, and weighed to the nearest 0.1 mg (Williams and Tieleman 2000; Liu and Li 2006; Wu et al. 2014).

Preparation of mitochondria

Liver and pectoral muscle sub-samples were placed in ice-cold, sucrose-buffered medium, cleaned of any adhering tissue, blotted, and weighed. We chopped liver samples coarsely with scissors, then rinsed and resuspended them in 5 volumes of ice-cold medium (Rasmussen et al. 2004). Pectoral muscle samples were coarsely chopped with scissors, and treated with proteinase for 5–10 min, after which the proteinase was removed and the muscle samples were resuspended in 10 volumes of ice-cold medium. Both liver and muscle preparations were homogenized with a Teflon/glass homogenizer. Homogenates were centrifuged at $600\times g$ for 10 min at 4 °C in an Eppendorf centrifuge, and the pellets containing nuclei and cellular debris discarded. Supernatants were centrifuged at $12{,}000\times g$ for 10 min at 4 °C. The resultant pellets were suspended, respun at $12{,}000\times g$, and resuspended (2:1, w/v for liver and 4:1 for muscle) in ice-cold medium (Zheng et al. 2013b). We determined the protein content of mitochondria by the Folin phenol method with bovine serum albumin as standard (Lowry et al. 1951).

Mitochondrial respiration and enzyme activity

Mitochondrial state-4 respiration in liver and pectoral muscle was measured at 30 °C in 1.96 mL of respiration medium with a Clark electrode (Hansatech Instruments LTD., England, DW-1), essentially as described by Estabrook (1967). State-4 respiration was measured over a 1 h period under substrate dependent conditions, with succinate as the substrate (Zheng et al. 2014a). State-4 respiration was expressed as mean mass-specific level [μmol O_2/(min g tissue)] (Zheng et al. 2013a). Cytochrome c oxidase (COX) activity in the liver and pectoral muscle was measured polarographically at 30 °C using a Clark electrode according to Sundin et al. (1987). Enzyme activity was reported as mean mass-specific level [μmol O_2/(min g tissue)] (Zheng et al. 2013b, 2014a).

Statistics

Statistical analyses were performed using the SPSS package (version 12.0). All variables were tested for normality with the Kolmogorov–Smirnov test before statistical tests were performed. Non-normal data were normalized by transforming them to their natural logarithms before conducting statistical tests. M_b among different groups was compared using a one-way ANOVA. The significance of differences in BMR and organ mass was determined with a one-way ANCOVA with M_b as a covariate. We used Tukey's HSD post hoc test to determine which species differed significantly from others. The statistical significance of differences in mitochondrial protein, mitochondrial state-4 respiration and COX activity in the liver and muscle was tested with a one-way ANOVA. Least-squares linear regression was used to evaluate the relationship between log BMR and log M_b, and between log BMR, log state-4 respiration and log COX. Data are reported as mean ± SE, unless otherwise noted. The p values <0.05 were considered statistically significant.

Results

Body mass (M_b) and basal metabolic rate

There were significant differences in M_bs among the three species ($F_{2,22} = 21.303$, $p < 0.001$; Fig. 1A; Table 1). There were also significant differences in BMR (mL O_2/h) among the three species ($F_{2,22} = 26.772$, $p < 0.001$; Fig. 1B); mean BMR was significantly higher in Eurasian Tree Sparrows than in Bramblings (19.0 %) and Little Buntings (74.4 %). Corrected for M_b, BMR still differed significantly among the three species ($F_{2,21} = 5.402$, $p < 0.05$, Table 1). There was a positive correlation between M_b and BMR ($r = 0.768$, $p < 0.001$; Fig. 1C). No significant differences were found between the three species in body temperature (data not shown).

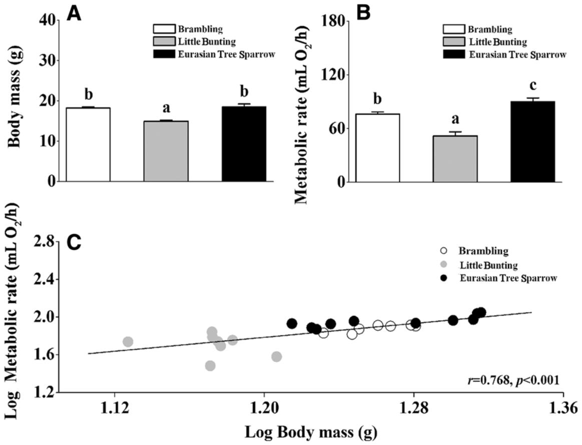

Fig. 1 Comparison of M_b (**A**), basal metabolic rate (**B**) and the relationship between log M_b and log basal metabolic rate (**C**) among Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*). Data are shown as mean ± SE, *bars* with *different letters* are significantly different

Organ and muscle mass

Gizzard mass differed significantly among the three species ($F_{2,21} = 8.852$, $p < 0.01$; Table 1). The mean gizzard mass of Eurasian Tree Sparrows was heavier than that of Bramblings, but there was no significant difference in gizzard mass between Eurasian Tree Sparrows and Little Buntings, or between that of Little Buntings and Bramblings. The three species also differed significantly in rectal mass ($F_{2,21} = 5.815$, $p < 0.01$; Table 1). Eurasian Tree Sparrows and Bramblings had a higher average rectal mass than Little Buntings, but there was no significant difference in rectal mass between Eurasian Tree Sparrows and Bramblings. Eurasian Tree Sparrows had a higher average total digestive tract mass than Bramblings and Little Buntings ($F_{2,21} = 21.358$, $p < 0.001$), but there was no significant difference in this variable between the latter two species ($p > 0.05$). No significant between-species differences were apparent in the dry mass of the heart, liver, kidneys, small intestine, or muscle (Table 1). Partial correlations between log organ mass and log M_b were positive for all organs, and the dry mass of the heart, liver, kidneys, gizzard, total digestive tract, and muscle, were significantly correlated with M_b (Table 2). For each of these organs, the slopes of the respective regression lines exceeded 1.0, indicating that organ mass increased with body size at a faster rate than overall M_b (Table 2). BMR residuals were only significantly, positively correlated with the total digestive tract dry mass residuals (Table 2).

Table 1 Comparison of the body mass, basal metabolic rate and internal organ dry mass among Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*)

	Fringilla montifringilla	*Emberiza pusilla*	*Passer montanus*	Significance
Sample size (n)	7	8	10	
Body mass (g)	18.2 ± 0.3^b	14.9 ± 0.3^a	18.6 ± 0.6^b	$F_{2,22} = 21.303, p < 0.001$
Basal metabolic rate [mL/(O_2·h)]	71.63 ± 3.90^a	63.36 ± 5.14^a	84.28 ± 3.68^b	$F_{2,21} = 5.402, p < 0.05$
Muscle (mg)	394.0 ± 19.9	391.1 ± 26.3	435.2 ± 18.8	$F_{2,21} = 1.598, p > 0.05$
Heart (mg)	50.8 ± 4.1	45.2 ± 5.3	59.9 ± 3.8	$F_{2,21} = 2.569, p > 0.05$
Liver (mg)	187.1 ± 16.8	234.1 ± 22.2	223.1 ± 15.9	$F_{2,21} = 2.082, p > 0.05$
Kidney (mg)	36.3 ± 2.2	36.2 ± 2.9	37.9 ± 2.1	$F_{2,21} = 0.175, p > 0.05$
Gizzard (mg)	77.6 ± 8.4^a	94.4 ± 11.0^{ab}	120.5 ± 7.9^b	$F_{2,21} = 8.852, p < 0.01$
Small intestine (mg)	72.3 ± 5.0	65.4 ± 6.6	71.5 ± 4.7	$F_{2,21} = 0.284, p > 0.05$
Rectum (mg)	5.9 ± 0.3^b	3.8 ± 0.4^a	5.2 ± 0.3^b	$F_{2,21} = 5.815, p < 0.01$
Digestive tract (mg)	155.7 ± 7.9^a	163.5 ± 10.5^a	197.1 ± 7.5^b	$F_{2,21} = 21.358, p < 0.001$

Statistical significance was determined by one-way ANCOVA with body mass as a covariate. Data are presented as mean ± SE. The different superscripts in the same row indicate significant differences

Table 2 Linear regression statistics for log dry organ mass versus log body mass (partial correlations) and log dry organ mass versus log BMR residuals among small birds in China

	Muscle	Heart	Liver	Kidney	Gizzard	Small intestine	Rectum	Digestive tract
Partial correlations								
R^2	0.669	0.193	0.002	0.397	0.406	0.038	0.076	0.487
p	*<0.01*	*<0.05*	*<0.05*	*<0.01*	*<0.001*	0.177	0.098	*<0.01*
Slope	1.292	0.898	0.070	1.030	1.720	0.408	0.635	1.226
Residual correlations								
R^2	0.008	0.020	0.019	0.043	0.058	0.056	0.020	0.169
p	0.664	0.235	0.513	0.949	0.129	0.133	0.235	*<0.05*
Slope	0.050	0.253	0.143	0.010	0.381	0.267	0.269	0.336

Values in italic indicate statistically significant results

Protein content, mitochondrial state-4 respiration, and cytochrome c oxidase (COX) activity in liver and muscle

There were no significant interspecific differences in the protein content of different organs (liver, $F_{2,22} = 1.007$, $p > 0.05$; muscle, $F_{2,22} = 0.360$, $p > 0.05$; Fig. 2A), but Eurasian Tree Sparrows had higher mitochondrial state-4 respiration (liver, $F_{2,22} = 4.374$, $p < 0.05$; muscle, $F_{2,22} = 15.108$, $p < 0.001$; Fig. 2B) and COX activity (liver, $F_{2,22} = 9.615$, $p < 0.01$; muscle, $F_{2,22} = 8.492$, $p < 0.01$; Fig. 2C) than Bramblings and Little Buntings. No significant differences were found in these variables between the latter two species ($p > 0.05$). Log BMR was positively correlated with log COX activity in the liver ($r = 0.388$, $p < 0.05$; Fig. 3b), log state-4 respiration ($r = 0.568$, $p < 0.01$; Fig. 3c) and log COX activity ($r = 0.548$, $p < 0.01$; Fig. 3d) in muscle.

Discussion

The results of this study indicate significant differences in organ mass, and some biochemical markers of metabolic tissue activity, among the three species, which could partly account for the observed interspecific differences in BMR (Guderley et al. 2005).

Comparison of internal organ mass and thermogenic properties

With respect to metabolic traits, the BMR of an animal is the sum of the metabolic rates of its organs and other metabolically active tissues (Zheng et al. 2008a; Swanson 2010; Clapham 2012). The selective pressures that influence metabolism may, however, be complex and act on metabolic rate through multiple avenues. Two of these potential avenues are to alter the sizes of tissues or organs and to alter the density of mitochondria and the

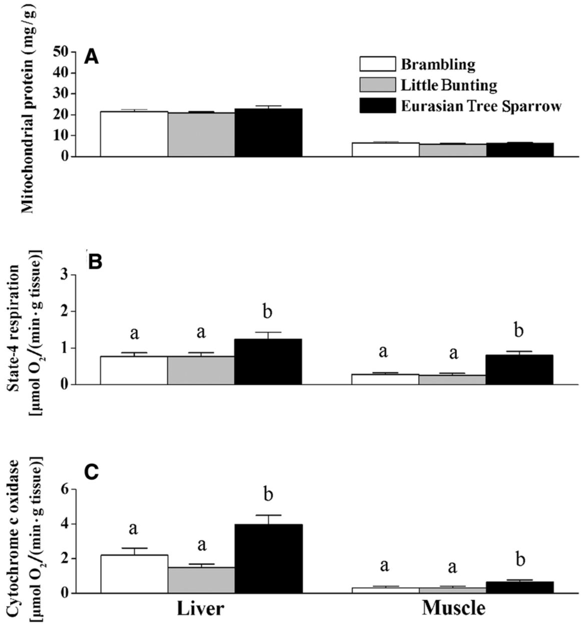

Fig. 2 Differences in mitochondrial protein (**A**), state-4 respiration (**B**), and cytochrome c oxidase (**C**) in the liver and pectoral muscle among Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*). *Data* are shown as mean ± SE, *bars* with *different letters* are significantly different

concentration of enzymes in aerobic catabolic pathways (Brand et al. 2003; Else et al. 2004). However, it is not clear whether large, energetically expensive organs are responsible for higher BMR, or whether they are necessary to support a higher BMR. Thus, the relationship between BMR and organ mass remains purely correlative, which is cause and which is effect remains unresolved (Steyermark et al. 2005). What are the ecological and evolutionary implications of having larger visceral organs for higher BMR birds? It has been suggested that much of the energy used in basal metabolism is consumed by the visceral organs (Daan et al. 1990; Piersma et al. 1996). Williams and Tieleman (2000) hypothesized that natural selection adjusts the size of the internal organs to match energy requirements, and that body size independent variation in BMR reflects the relative size of internal organs. These include the digestive tract, which performs digestion and absorption, the heart, which transports oxygen to the tissues, the liver which performs catabolism, and the kidneys, which eliminate nitrogenous and other wastes (Kersten and Piersma 1987; Daan et al. 1990; Hammond et al. 2001). We found no significant interspecific differences in heart, liver, kidneys, or muscle mass, and consequently no evidence to support the hypothesis that the mass of these organs should be greater in species with higher BMR. However, compared to Bramblings and Little Buntings, Eurasian Tree Sparrows had a heavier gizzard, rectum and total digestive tract. These findings suggest that the mass of the digestive organs could be related to the observed between-species differences in BMR. The ecological implications of having a larger digestive tract are increased food consumption, which could, in turn, stimulate the enlargement of organs such as the gizzard and small intestine (Zheng et al. 2008b; Lv et al. 2014). For example, Zheng et al. (2013b) acclimated Chinese Bulbuls (*Pycnonotus sinensis*) to either 10 or 30 °C for 4 weeks, measured their BMR, and then determined the dry mass of their internal organs. Bulbuls acclimated to 10 °C had a significantly higher BMR, and a markedly larger liver and intestine than those acclimated to 30 °C. Eurasian Tree Sparrows also had a significantly higher BMR in winter than in summer, and had a larger liver, smaller intestine and entire digestive tract compared to birds examined in summer (Liu and Li 2006; Zheng et al. 2008b). Changes in the size of digestive organs in response to elevated daily energy intake could therefore result in elevated BMR (Williams and Tieleman 2000).

Comparison of biochemical indices and thermogenic properties

Interspecific differences in metabolic intensity are linked with differences in mitochondrial densities, oxidative capacities and mitochondrial proton leaks (Else et al. 2004; Guderley et al. 2005). A strong correlation between metabolic rate, mitochondrial respiration, and proton leaks has been reported (Brookes et al. 1998; Li et al. 2010). The liver is one of the largest, and most metabolically active, organs in endotherms, and is considered to make an important contribution to BMR (Villarin et al. 2003; Zheng et al. 2008b). Mechanisms of heat generation in the liver include the uncoupling of oxidative phosphorylation, futile cycling of substrates and high mass-specific metabolic intensity (Brand et al. 2003; Zheng et al. 2014a). For example, Else et al. (2004) compared the respiration rate of hepatocytes in five birds and found that these approximated the basal metabolic rate–body mass relationship. Similar results have also been obtained in small mammals. For example, in addition to higher BMR, Brandt's Voles (*Lasiopodomys brandtii*) also had higher mitochondrial state-4 respiration capacity and COX activity in the liver than Mongolian Gerbils (*Meriones unguiculatus*), suggesting that there is a relationship between these metabolic process and BMR (Li et al. 2010). In the present study, we found significant interspecific differences in state-4 respiration and COX activity in the liver, and significant, positive correlations between BMR and COX activity. These results suggest that the higher metabolic activity in the liver of Eurasian

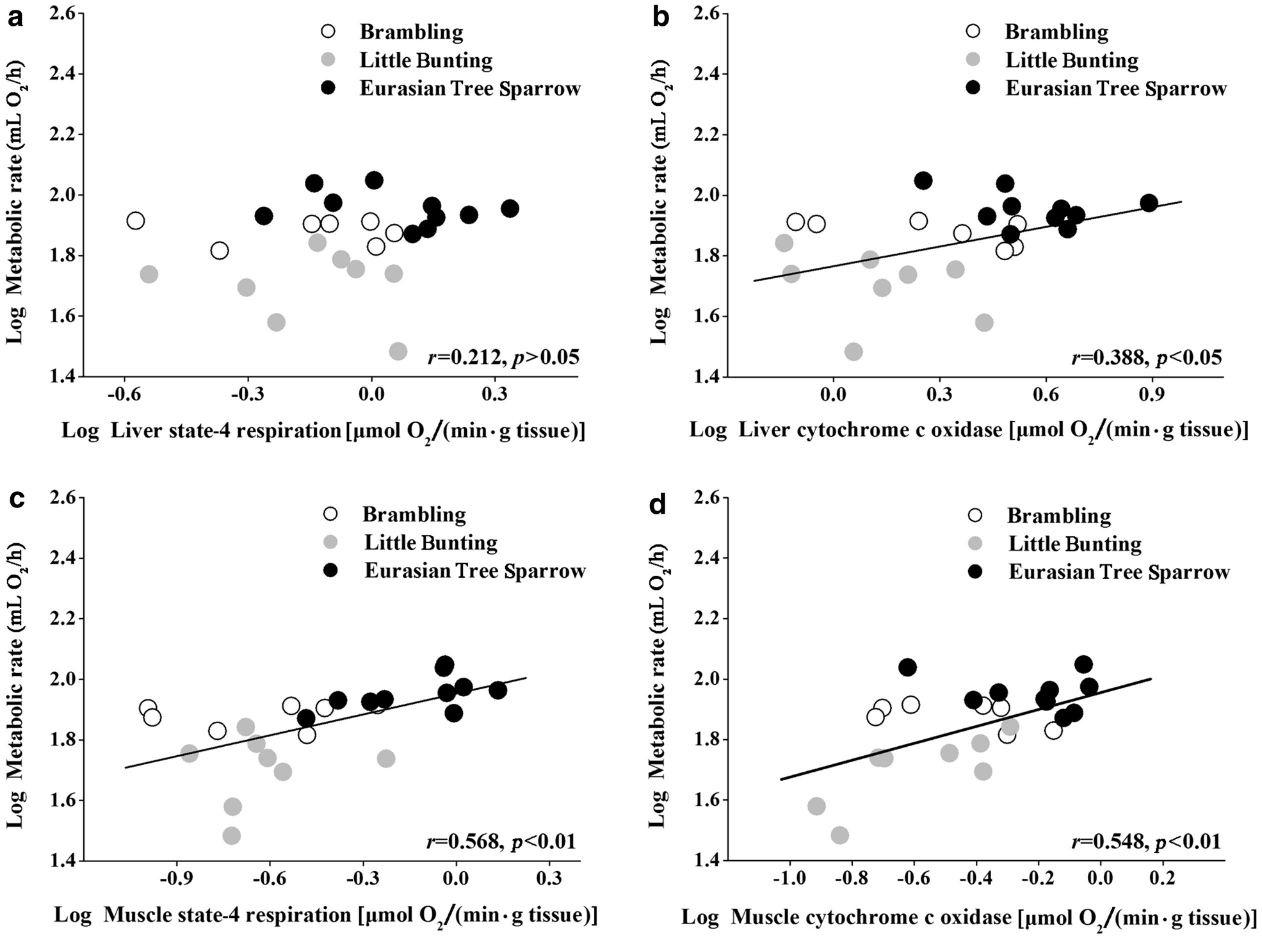

Fig. 3 Correlations between log metabolic rate and state-4 respiration in the liver (**a**), and cytochrome c oxidase (COX) activity in the liver (**b**), state-4respiration in pectoral muscle (**c**) and cytochrome c oxidase (COX) activity in pectoral muscle (**d**), among Bramblings (*Fringilla montifringilla*), Little Buntings (*Emberiza pusilla*) and Eurasian Tree Sparrows (*Passer montanus*)

Tree Sparrows may contribute to the observed interspecific differences in BMR. This finding is in agreement with the results of our previous studies which show that seasonal and latitudinal variation in Eurasian Tree Sparrows was correlated not only with variation in BMR, but also in state-4 respiration and COX activity in the liver (Zheng et al. 2008b, 2014b).

Because skeletal muscle mass comprises nearly 40 % of M_b, it is an important contributor to thermogenesis via shivering, and even nonshivering thermogenesis (Bicudo et al. 2001; Pitit and Vézina 2014). Furthermore, adjustment of cellular aerobic capacity in muscle potentially involves modulation of the activities of key catabolic enzymes in oxidative pathways, and, or, the activities of enzymes and transporters involved in substrate mobilization and delivery pathways (Marsh et al. 1990; Swanson 2010; Zheng et al. 2008b, 2014a). The results of this study demonstrate that Eurasian Tree Sparrows had higher mitochondrial state-4 respiration and COX activity than Bramblings and Little Buntings, and that there was a positive relationship between BMR, state-4 respiration and COX activity in these three species. This suggests that biochemical metabolic markers may be useful indicators of interspecific variation in BMR.

Conclusions

The selective pressures that influence metabolism may be complex and influence metabolic rate via multiple avenues. Our results show that Eurasian Tree Sparrows had significantly higher BMR, digestive organ mass, mitochondrial state-4 respiration capacity and COX activity in the liver and muscle, than Bramblings and Little Buntings. This suggests that digestive organ mass and the above biochemical markers of metabolic activity are both strongly correlated with BMR in these species, and play an important role in the determination of BMR. Future studies could add to these results by measuring thyroid hormones (thyroxine and triiodothyronine), which affect adaptive thermogenesis by substrate cycling, ion cycling, and mitochondrial proton leakage (Yen 2001; Liu et al. 2006; Mullur et al. 2014). Additional avenues for further research on the mechanisms underlying BMR variations include quantifying inter- and intraspecific variation in avian uncoupling protein (avUCP), proton conductance, and myostatin, all of which can affect the basal thermogenesis of tissues (Dridi et al. 2004; Swanson 2010).

Authors' contributions

JL provided the research idea and designed the experiments. MB, XU and KC conducted the experiments and collected the data. MB and XU finished the data analysis, compiled the results and wrote the first draft of the article. JL and WZ supervised the research and revised the draft. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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