Geographic variation in the response to thermal acclimation in rufous-collared sparrows: are physiological flexibility and environmental heterogeneity correlated?

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Summary

1. It has been proposed that habitats with low productivity, and hence low resource availability, may favour individuals with lower rates of resource use and energy expenditure. In birds, some studies have shown that basal metabolic rate (BMR) and total evaporative water loss (TEWL) are reduced in desert bird species compared to those that live in more mesic areas. However, the contribution of the phenotypic plasticity of birds to this pattern has been poorly studied.
2. We evaluated the phenotypic flexibility of BMR, TEWL and organ size in sparrows (*Zonotrichia capensis*) from three populations along an aridity gradient that differ in environmental temperature, rainfall and seasonality. We also tested the hypothesis that populations from variable environments exhibit more physiological flexibility than populations from stable environments.
3. BMR and TEWL in *Z. capensis* exhibited a positive association with latitude. Additionally, the population from the more stable environment was unaffected by thermal acclimation, but birds from seasonal environments were affected.
4. Our results support the hypothesis of the existence of a correspondence between environmental variability and phenotypic flexibility; that is, birds from localities with a drastic climatic seasonality have a greater response in BMR to thermal acclimation than birds from a more stable environment.

Key-words: aridity gradient, BMR, phenotypic flexibility, TEWL, *Zonotrichia capensis*

Introduction

Inter-specific comparisons have demonstrated that bird species that inhabit arid environments with high temperatures, low relative humidity and low primary productivity, show physiological adjustments such as reduced basal metabolic rate (BMR) and total evaporative water loss (TEWL) in comparison with species that live in mesic environments (Hudson & Kimzey 1966; Withers & Williams 1990; Hinsley *et al*. 1993; Tieleman & Williams 1999; Tieleman, Williams & Buschur 2002a). It was suggested that low productivity, and hence low resource availability, might favour individuals with lower rates of resource use and energy expenditure. In addition, scarcity of water in xeric areas could be a selective

pressure that favours reduced metabolic rates, because the resultant low endogenous heat production may diminish the water requirements for evaporative cooling (Dawson 1984).

Tieleman, Williams & Bloomer (2002b) studied several bird species of the Alaudidae family and incorporated environmental gradients and phylogenetic history in their statistical analysis, finding a high correlation between an aridity index and physiological traits. On the other hand, McKechnie, Freckleton & Jetz (2006) found a significant signal of phenotypic plasticity in avian metabolic scaling, and called for more studies that partition the variation of physiological traits into genetic and phenotypic components. Few studies on the physiological diversity of birds have partitioned phenotypic variation (Wikelski *et al*. 2003; Broggi *et al*. 2005), and hence how much of the variability in BMR and other physiological features related to habitat in birds has a genetic basis, or is attributable to phenotypic plasticity, remains unknown.

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Pigliucci (2001) proposed that phenotypic plasticity is an important component of the ecology of organisms and that there are associated energetic costs of maintenance and production of plastic structures. Intra-individual phenotypic flexibility (sensu Piersma & Drent 2003) is expected to be large in species from temporally heterogeneous environments where the ecological situations change in the course of an individual's life. Hence, the maintenance of inter-individual variation among phenotypes appears dependent upon the frequency of environmental change and on the spatial or temporal nature of environmental heterogeneity. Spatial heterogeneity can maintain genetic variation and temporal heterogeneity favours phenotypic plasticity, both potentially resulting in phenotypic variation (Hedrick 1986; Schlichting & Pigliucci 1998).

Williams & Tieleman (2000) proposed that the phenotypic flexibility in BMR and TEWL among desert birds will be greater than that among similar birds that live in mesic areas. The rationale for this hypothesis is that birds dwelling in habitats with low predictability of rain pulses and hence in resource abundance, will exhibit adjustments in their organ sizes and then in BMR. In an attempt to examine if desert birds might be more flexible than mesic species, Tieleman *et al*. (2003) tested if birds inhabiting unpredictable desert habitats have evolved higher physiological flexibility than more mesic species. The authors found inter-specific variation in metabolic responses to acclimation, but this was not consistently related to habitat aridity. However, in this study, it was implicitly assumed that more arid habitats would have higher variability in physical variables that determine directly (temperature and rainfall) and indirectly (via effects on productivity) BMR and TEWL, but the authors did not provide any data supporting the suggestion that more arid environments are more variable. In fact, it is likely that some mesic areas would be more predictable in rainfall, but presenting seasonal oscillations that maintain the flexibility in thermogenic traits. We hypothesize that the variation at the intra-specific level (i.e. physiological flexibility) would be correlated with the magnitude of seasonal variation in temperature and rainfall, but not with aridity or predictability of habitats *per se*. In addition, because in that study the species compared belonged to the same family but different genera, it is possible that the response to acclimation may be influenced by the putative effect of the phylogenetic origin. One avenue to diminish the confounding effect of phylogeny is to test for differences among populations of the same species.

The objective of this study was to examine the variability of BMR and TEWL among populations of the same species, and to assess how much of the physiological differences may be explained by phenotypic plasticity. Also, we tested the hypotheses of a functional correlation between ecological flexibility and phenotypic plasticity at an intra-specific level. We predicted that the magnitude of the adjustments in BMR and TEWL would depend on the degree of temporal heterogeneity (e.g. climatic seasonality) of the environment. We expected that populations from highly seasonal mesic environments would exhibit greater physiological flexibility

Table 1. Geographical origin and climatic data for three populations of rufous-collared sparrows in Chile*

| | Precipitation | | Temperature | | | |
	Annual	$\sigma^2 P$	Annual mean	σ^2 T	DMi	σ^2 DMi
Copiapo	11·2	2·2	15·7	10·1	0·04	0·004
Santiago	337·4	890	13·8	20·3	1·44	2·75
Valdivia	2441·2	1 6376	11·4	11	10·5	60·07

The de Martonne (DMi) aridity index was calculated following di Castri and Hajek (1976), based on average of monthly temperatures and precipitations. The mean annual temperature (calculated as the average of the monthly temperatures) and total annual precipitations are reported. The variances of precipitation ($\sigma^2 P$) temperature ($\sigma^2 T$) and de Martonne index (σ^2 DMi) were estimated from the mean of monthly values.
*Climatic data were collected from <http://www.worldclimate.com>.

than populations from more stable semi-arid zones. To test our hypothesis, we studied three populations of *Zonotrichia capensis* in Chile that experienced strong differences in climatic seasonality, temperature and rainfall regime (Table 1). Also, in this species it has been previously reported that TEWL and BMR vary between xeric and mesic environments (Sabat *et al*. 2006).

Materials and methods

SPECIES AND STUDY SITE

Animals were collected between November 2005 and February 2006 at three sites along a latitudinal gradient comprising more than 1500 km and more than three orders of magnitude of precipitation difference (di castri & Hajek 1976). The localities were (i) Copiapo (27°21′ S, 70°24′ W), (ii) Quebrada de la Plata (33°27′ S, 70°42′ W), in Santiago, and (iii) Valdivia (39°48′ S, 73°14′ W). Our study sites vary not only in rainfall and temperature, but also in the length of the season during which each site can be considered arid. Copiapo are dry year round, in Quebrada de la Plata dry season extends only from November to March, whereas Valdivia are rainy year round (di Castri & Hajek 1976). After capture, birds were transported to the laboratory in Santiago, (33°27′ S, 70°42′ W) and housed in individual $35 \times 35 \times 35$ cm plastic mesh cages. Temperature and photoperiod were maintained at 22 ± 2 °C and 12L : 12D, respectively. Birds had *ad libitum* access to mealworms, seeds and water. After 2 days of habituation to laboratory conditions, individuals were randomly assigned to one of two thermal treatments; one group was maintained at constant ambient temperatures (T_a) of 15 ± 3 °C and the second at T_a of 30 ± 3 °C with a similar light : dark cycle of 12L : 12D for 30 days. Water and food (adult larvae of mealworms, seed and boiled egg) were supplied *ad libitum*.

BASAL METABOLIC RATE (BMR) AND TOTAL EVAPORATIVE WATER LOSS (TEWL)

We measured rates of oxygen consumption (VO_2) and TEWL in post-absorptive, resting birds in the inactive phase, using standard flow-through respirometry and hygrometry methods. Inside dark metabolic chambers, birds perched on a wire-mesh grid that allowed

excreta to fall into a tray containing mineral oil, thus trapping the water from this source. Oxygen consumption was measured using a computerized, open-flow respirometry system (Sable Systems, Henderson, NV) calibrated with a known mix of oxygen (20%) and nitrogen (80%) that were certified by chromatography (INDURA, Chile). Measurements were made at ambient temperatures (T_a) of $25 \cdot 0$ and $30 \cdot 0 \pm 0 \cdot 5$ °C at random. We are confident that a T_a of $30 \cdot 0$ °C is within the thermo neutral zone for this species (Sabat *et al.* 2006). The metabolic chamber received dried air at 500 mL min^{-1} from a mass flow controller and through Bev-A-Line tubing (Thermoplastic Processes Inc., Warren, NJ). This flow ensured adequate mixing in the chamber. The mass flow meter was calibrated monthly with a volumetric (bubble) flow meter. The excurrent air passed through a RH-200 relative humidity/dewpoint hygrometer (Sable Systems) and through columns of Diedrite, CO_2-absorbent granules of Baralyme, and Drierite before passing through an O_2-analyser; model FC-10A (Sable Systems). The complete VO_2 trial lasted 3 h. Output from the H_2O (kPa) and oxygen analysers (%) was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 5 s. Birds remained in the chamber for at least 3 h and visual inspection of the recorded data allowed us to determine when steady-state conditions had been achieved. We averaged water vapour pressure and O_2 concentration of the excurrent airstream over a 20-min period after steady-state was reached (following Tieleman *et al.* 2002a). Because CO_2 was scrubbed before entering the O_2 analyser, oxygen consumption was calculated as $VO_2 = [FR \times 60 \times (F_iO_2 - F_eO_2)]/(1 - F_iO_2)$, where FR is the flow rate in millilitres per minute after STP correction, F_i and F_e are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. TEWL was calculated as TEWL $= [(V_e\rho_{out} - V_i\rho_{in})]$ where TEWL is in milligrams per millilitre. ρ_{in} and ρ_{out} are the absolute humidity in kilograms per cubic metre of the inlet air and the outlet air, respectively, V_e is the flow rate of the air entering the chamber as given by the mass flow controller (500 mL min^{-1}) and V_e is the flow of exiting air. V_e was calculated following Williams & Tieleman (2000) as $V_e = V_i - [VO_2(1 - RQ)] + VH_2O$; V_i, and VO_2 (mL min^{-1}) are known. We assumed a respiratory quotient (RQ) of $0 \cdot 71$. Absolute humidity was calculated as $\rho = P/(T \times R_w)$, where P is water vapour pressure of the air in Pascals, T is the temperature of the dew-point hygrometer in degrees Kelvin and R_w is the gas constant for water vapour ($461 \cdot 5$ J kg^{-1} · K, Lide 2001). The P_{in} was determined using the average value of the vapour pressure of the air entering the empty chamber (i.e. the baseline period of 15 min) before and after each experiment. Body mass was measured before the metabolic measurements using an electronic balance ($\pm 0 \cdot 1$ g) and cloacal body temperature (T_b) was recorded at the end of each measurement with a Cole–Palmer copper–constantan thermocouple attached to an Digi-Sense thermometer (Model 92800-15). Additionally we estimated the metabolic water production (MWP) of birds using equivalence: $0 \cdot 567$ mL H_2O L^{-1} consumed (Schmidt-Nielsen 1997); the ratio between MWP and TEWL was then calculated.

After metabolic experiments, birds were killed by exposure to CO_2 and dissected abdominally. We extracted the large and small intestine, and then heart, lungs, liver and kidneys. Fresh organ mass was determined immediately ($\pm 0 \cdot 05$ g).

DATA ANALYSIS

We estimated the environmental heterogeneity of each locality using the Martone aridity index (DMi), and calculating the annual variance of the index. DMi was calculated monthly as $DM = P/T + 10$, where P is the monthly precipitation (mm), T is the average monthly temperature (°C). Although not perhaps intuitively straightforward, this index was empirically derived to describe primary productivity in arid and semi-arid areas. The index is low in hot, dry deserts and high in cool, wet areas. Also the variance in DMi and hence the seasonal differences in temperature and precipitations are significantly lower in desert areas than in more mesic localities (see Table 1). Climatic data were collected from <http://www.worldclimate.com>. We evaluated the effect of m_b on physiology and morphology by means of a regression analysis using BMR, TEWL and organ masses as dependent variables and m_b as the independent variable. To test the effect of the locality and acclimation condition on TEWL and VO_2, we performed an analysis of covariance (ANCOVA), using body mass (m_b) as the covariate. Since some morphological variables were uncorrelated with body mass, we used an ANOVA using the locality and temperature of acclimation as fixed factors. To test for specific differences among means in physiological and morphological traits we used a *post hoc* Fisher test. In order to assess the association between BMR, TEWL and body mass, a linear regression analysis between the residuals of BMR and TEWL against body mass was performed. Since the analysed variables exhibited high co-linearity, we performed a principal components analysis (PCA) and then an ANOVA on the factor scores generated by PCA. Statistical analyses were performed using the STATISTICA® (1997) statistical package for Windows. Data are reported as mean $\pm$ SE.

Results

BASAL METABOLIC RATE (BMR) AND TOTAL EVAPORATIVE WATER LOSS (TEWL)

We found a significant and positive association between TEWL and BMR with body mass when all data were used (BMR, $r^2 = 0 \cdot 4$, $F_{(1,43)} = 34 \cdot 62$, $P < 0 \cdot 001$ and TEWL $r^2 = 0 \cdot 3$, $F_{(1,45)} = 21 \cdot 32$, $P < 0 \cdot 001$). The analysis of the residuals of BMR and TEWL (from linear regressions with m_b) exhibited a significant and positive relationship ($r^2 = 0 \cdot 31$, $F_{(1,43)} = 20 \cdot 03$, $P < 0 \cdot 001$). *Zonotrichia capensis* exhibited significant adjustments of BMR in response to thermal acclimation. Cold-acclimatized birds had higher values of BMR than warm-acclimated birds ($82 \cdot 68 \pm 2 \cdot 50$ mL O_2 h^{-1}, $n = 23$ and $47 \pm 2 \cdot 26$ mL O_2 h^{-1}, $n = 22$; for cold and warm groups, respectively; $F_{(1,38)} = 7 \cdot 31$, $P = 0 \cdot 01$). Additionally we found that sparrows from different localities exhibited differences in BMR ($F_{(2,38)} = 3 \cdot 4$, $P = 0 \cdot 04$), but the interaction was not significant ($F_{(2,38)} = 1 \cdot 16$, $P = 0 \cdot 19$). In addition, we found that the acclimation response of the three different populations varied. The *a posteriori* test revealed that the BMR of birds from Copiapo was unaffected by thermal acclimation ($P = 0 \cdot 48$), whereas BMR values in birds from Santiago and Valdivia increased significantly after the cold treatment (Fig. 1). However, the BMR of sparrows from Copiapo acclimated to 15 °C was significantly lower than that of birds from Santiago ($P < 0 \cdot 005$) and Valdivia ($P < 0 \cdot 001$), acclimated to 30 °C.

The origin of birds also had a significant effect on TEWL. The values of TEWL in birds from Copiapo were significantly lower than those found in sparrows from Santiago and Valdivia ($F_{(2,38)} = 15 \cdot 6$, $P < 0 \cdot 01$). TEWL in birds from Valdivia was

Table 2. Body mass and wet organ mass (g) in *Z. capensis* from different localities acclimated to one of two contrasting temperatures

	Copiapo		Santiago		Valdivia	
	15 °C (6)	30 °C (7)	15 °C (10)	30 °C (9)	15 °C (7)	30 °C (6)
mb (g)	$20\cdot35 \pm 0\cdot60^a$	$19\cdot96 \pm 0\cdot49^a$	$20\cdot4 \pm 0\cdot59^a$	$20\cdot81 \pm 0\cdot15^a$	$20\cdot81 \pm 0\cdot38^b$	$22\cdot34 \pm 0\cdot85^b$
Kidney (g)	$0\cdot20 \pm 0\cdot005^a$	$0\cdot19 \pm 0\cdot01^a$	$0\cdot28 \pm 0\cdot01^c$	$0\cdot23 \pm 0\cdot01^{a,d}$	$0\cdot26 \pm 0\cdot01^{b,c}$	$0\cdot24 \pm 0\cdot01^{b,d}$
Liver (g)	$0\cdot53 \pm 0\cdot20^{a,b}$	$0\cdot47 \pm 0\cdot02^{a,b}$	$0\cdot76 \pm 0\cdot06^c$	$0\cdot53 \pm 0\cdot05^{b,d}$	$0\cdot94 \pm 0\cdot06^{a,d}$	$0\cdot62 \pm 0\cdot02^{c,e}$
Intestine (g)	$0\cdot69 \pm 0\cdot04^{a,c}$	$0\cdot62 \pm 0\cdot01^a$	$0\cdot10 \pm 0\cdot05^{b,c}$	$1\cdot07 \pm 0\cdot25^b$	$1\cdot09 \pm 0\cdot06^b$	$0\cdot90 \pm 0\cdot06^{a,b}$
Heart (g)	$0\cdot26 \pm 0\cdot02^a$	$0\cdot26 \pm 0\cdot01^a$	$0\cdot28 \pm 0\cdot01^b$	$0\cdot23 \pm 0\cdot01^c$	$0\cdot31 \pm 0\cdot01^b$	$0\cdot29 \pm 0\cdot01^b$
Gizzard (g)	$0\cdot54 \pm 0\cdot07^a$	$0\cdot56 \pm 0\cdot02^a$	$0\cdot88 \pm 0\cdot05^b$	$0\cdot73 \pm 0\cdot05^{a,b}$	$0\cdot84 \pm 0\cdot06^b$	$0\cdot70 \pm 0\cdot02^{a,b}$

Difference letters denote significant differences between temperatures and the number of animals of each treatment is in parenthesis. Data are reported as mean ± SE.
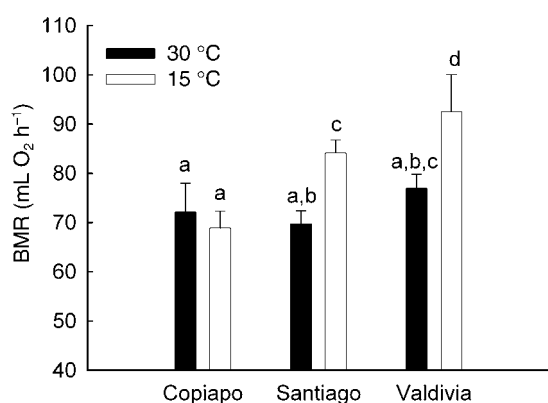

Fig. 1. BMR of *Z. capensis* from three populations in Chile, acclimated to two contrasting temperatures. Letters denote significant differences between temperatures. Data are reported as mean ± SE.
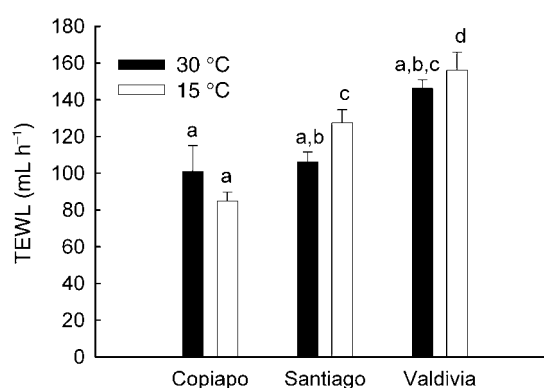

Fig. 2. TEWL of *Z. capensis* from three populations in Chile, acclimated to two contrasting temperatures. Letters denote significant differences between temperatures. Data are reported as mean ± SE.

29% and 62% higher than in those of in Copiapo and Santiago, respectively. TEWL was affected neither by thermal acclimation ($F_{(1,38)} = 0\cdot04$, $P = 0\cdot82$), nor by the interaction of acclimation and locality ($F_{(2,38)} = 1\cdot4$, $P = 0\cdot25$). However, the *a posteriori* analysis revealed that the sparrows from Santiago acclimated to 15 °C increased their TEWL significantly (20%), compared with values from the warm treatment (Fig. 2).

MORPHOLOGY

We found a significant association between body mass and kidney (kidney $r^2 = 0\cdot13$, $F_{(1,43)} = 6\cdot62$, $P = 0\cdot014$) and heart ($r^2 = 0\cdot20$, $F_{(1,43)} = 10\cdot92$, $P = 0\cdot002$) mass. Comparisons of fresh organ masses between acclimation treatments are shown in Table 2. Significant differences were found in kidney (ANCOVA, $F_{(1,38)} = 7\cdot67$, $P = 0\cdot008$) and liver (ANCOVA, $F_{(1,39)} = 12\cdot09$, $P = 0\cdot001$. We did not find significant differences between acclimation treatments in fresh mass of heart (ANCOVA, $F_{(1,38)} = 2\cdot8$, $P = 0\cdot1$), gizzard (ANCOVA, $F_{(1,39)} = 2\cdot35$, $P = 0\cdot1$), nor small intestine (ANCOVA, $F_{(1,39)} = 0\cdot4$, $P = 0\cdot5$). In addition, we found differences between localities for kidney ($F_{(1,38)} = 12\cdot23$, $P < 0\cdot001$) heart ($F_{(1,38)} = 3\cdot75$, $P = 0\cdot03$), small intestine ($F_{(2,39)} = 6\cdot4$, $P = 0\cdot003$) and gizzard ($F_{(2,39)} = 8\cdot2$, $P = 0\cdot001$). We did not find significant differences in the

interaction between treatment and locality (kidney $F_{(2,38)} = 2\cdot67$, $P = 0\cdot08$; heart $F_{(2,38)} = 1\cdot87$, $P = 0\cdot1$; gizzard $F_{(2,39)} = 0\cdot86$, $P = 0\cdot42$; liver $F_{(2,38)} = 1\cdot36$, $P = 0\cdot26$ or small intestine $F_{(2,39)} = 0\cdot73$, $P = 0\cdot5$).

Because of the high colinearity among the predictor variables, we performed a PCA, including all variables in the model. The results of this analysis revealed that the nine variables in the model were reduced to two PCA axes, which accounted for 70·37% of the variance (Table 3). The first component axis (PCA1) was strongly positively correlated with TEWL, BMR and body mass; and the second axis (PCA2) was significantly correlated with liver mass, gizzard mass and intestine mass. The first axis may be interpreted as a variable related to energetic expenditure and the second axis as a variable related to the ingestion and processing of matter and energy. The ANOVA revealed a strong effect of locality ($F_{(2,39)} = 13\cdot41$, $P < 0\cdot0001$) and acclimation ($F_{(1,39)} = 7\cdot89$, $P < 0\cdot01$) on the first axis. However, we did not find a significant effect in the interaction between treatment and locality ($F_{(2,39)} = 0\cdot58$, $P = 0\cdot56$). The second axis was affected by locality ($F_{(2,39)} = 6\cdot95$, $P < 0\cdot01$) but not by the acclimation ($F_{(1,39)} = 2\cdot27$, $P = 0\cdot13$). The interaction term was near the limit of significance ($F_{(2,39)} = 2\cdot84$, $P = 0\cdot07$). The *post hoc* test revealed that both axes increased in cold-acclimated birds

Table 3. PCA axis derived from analysis of energetic features in *Z. capensis*

Variables	PCA axis 1	PCA axis 2
Factor loadings		
TEWL	0·78	0·34
Body mass	0·78	0·19
BMR	0·90	0·16
Kidney mass	0·45	0·65
Liver mass	0·37	0·75
Gizzard mass	0·16	0·89
Intestine mass	0·19	0·83
Heart mass	0·68	0·27
Eigenvalue	4·39	1·23
Explained variance (%)	54·25	15·4
Explained variance cumulative (%)	54·95	70·37
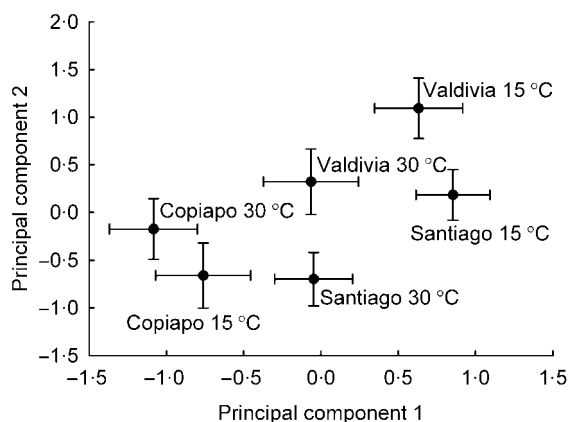
Fig. 3. Results of principal components analysis (PCA) on morphological and physiological variables studied in *Z. capensis* from three localities of Chile acclimated either to warm or cold temperature. The PCA1 was strongly positively correlated with TEWL, BMR and body mass; and the PCA2 was significantly correlated with liver mass, gizzard mass and intestine mass.

from Santiago and Valdivia, whereas they remained unchanged in sparrows from Copiapo (Fig. 3).

Discussion

Classical comparative studies have demonstrated that both BMR and TEWL are reduced in desert birds species compared to ones that live in more mesic areas (Dawson & Bennett 1973; Weathers 1979; Arad & Marder 1982; Withers & Williams 1990; Schleucher, Prinzinger & Withers 1991); and that they vary along aridity gradients (Nagy, Girard & Brown 1999; Williams & Tieleman 2001; Tieleman *et al.* 2002b). Our results agree with previous studies that have documented similar patterns at the inter-specific (Williams 1996; Tieleman & Williams 2000) and intra-specific (Hudson & Kimzey 1966) levels in birds. The values of BMR and TEWL in *Z. capensis* exhibited a positive association with latitude: birds from the semi-arid locality had BMR values which averaged 9·5% lower than those of birds from Santiago and 20% lower than those from Valdivia. Similarly, values of TEWL were on the

average 29% and 62% lower in sparrows from Copiapo than those of sparrows from Santiago and Valdivia, respectively. Our results support the general idea that environments with high environmental temperatures, low water and probably low food availability favour phenotypes with lower energetic requirements and lower rate of evaporative water loss.

BMR appears to be a plastic character that not only varies among populations, but also at the individual level (Bech, Langseth & Gabrielsen 1999; Horak *et al.* 2002; Labocha *et al.* 2004). In accordance, in this study we found some significant adjustments of BMR and also of TEWL to thermal acclimation. The values of BMR and TEWL in *Z. capensis* were higher in cold-acclimated birds than in warm-acclimated birds from the more seasonality localities, Santiago and Valdivia. Also, coupled with the metabolic response to the cold-acclimation, we found a substantial increase in liver and kidney mass (Table 2). Our results are consistent with the 'energy demand' hypothesis for short-term adjustments, which states that birds may adjust the size of their internal organs in synchrony with energy demands (see Williams & Tieleman 2000). *Zonotrichia capensis* exhibited differences in the mass of organs, which have been reported to have comparatively high mass-specific metabolic rates (e.g. liver, heart, gizzard, intestine and kidney), increasing along the latitudinal gradient. The analysis of principal components allowed us to analyse the response to acclimation in an integrated way. The first axis, which was strongly and positively correlated with energy expenditure (TEWL, BMR and body mass) varies in the same direction and magnitude as the second axis, which was strongly correlated with morphological variables related to the capacity of acquisition and processing matter and energy (liver mass, gizzard mass and intestine mass). These results support the hypothesis that the need to respond to high thermoregulatory demands occurs coupled with an increase in the capacity of acquisition, processing and energy expenditure (Williams & Tieleman 2000; Bacigalupe & Bozinovic 2002; Tieleman *et al.* 2003). Besides it has been documented that the rise of metabolic rate may also be associated with increase in the specific metabolic rate of different tissues (Krebs 1950; Daan, Masman & Groenewold 1990; Williams & Tieleman 2000; Vezina & Williams 2005). Future studies are necessary to determine if differences in BMR of natural populations may be the result of modifications in the size and/or the specific metabolic activity of the internal organs.

Recently Klaassen, Oltrogge & Trost (2004) proposed that the phenotypic plasticity of the characteristics related to energetic expenditure might be common to all birds. In contrast, Schlichting & Pigliucci (1998) proposed that phenotypic plasticity should be limited to specialist individuals and that it should have an adaptive value in organisms that experience temporary variations in their biotic and physical environment. Our results support these last hypotheses: the three populations of *Z. capensis* had different responses to thermal acclimation. Also, the magnitude of the plastic response to thermal acclimation was coupled with environmental temporal heterogeneity. In fact, BMR and TEWL of birds from Copiapo (the semi-arid locality) were not affected

by thermal acclimation, but they were affected in birds from mesic and more seasonal environments. It is probable that the semi-arid environmental condition of Copiapo, characterized by with high mean temperatures and low water availability, coupled with a lower physical environment variability, has exercised in the past a large selective pressure, favouring reduced metabolic and water loss rates and fixing these traits in the population.

On the other hand, our results do not support the hypothesis proposed by Williams & Tieleman (2000) that environmental aridity would select for physiological flexibility. Indeed, we found the opposed pattern, that is greater phenotypic flexibility in populations from more mesic sites. It appears that the failure to find a correlation between environment and physiological flexibility in previous studies may be due to the lack of proper assessment of environmental variability. For example, Tieleman *et al*. (2003) did not find support for such hypothesis in lark species dwelling along an aridity gradient. In their study the arid environments are assumed to be temporally more heterogeneous and unpredictable than mesic sites. However, at least one of those mesic sites (e.g. Northern Cape, South Africa) can be considered temporary hetero-geneous, because it exhibits pronounced seasonal climatic fluctuations (see Williams, Anderson & Richardson 1997). Moreover, compared with our semi-arid locality, the desert locality where larks were studied present higher seasonality in precipitation (absent in summer to ca. 50–250 mm in winter, see Williams *et al*. 2002) than the semi-arid locality of Copiapo, which is almost dry year round (only 11 mm in winter months). This difference in the degree of seasonality between those environments may explain in part the differences found in the response to acclimation between larks and *Z. capensis*. Besides, in the present study, the semi-arid locality of Copiapo could be considered unpredictable, because precipitations are very scarce and concentrated in winter. In the other extreme, Valdivia exhibits higher predictability, characterized by rains and cool temperatures year round. Because *Z. capensis* from Copiapo did not exhibits physiological flexibility whereas those from Valdivia did, we found no evidence to support the hypothesis that the unpredictability of habitats would select for physiological flexibility. We suggest that the magnitude of seasonality in temperature and rainfall are the physical variables explaining the intra-individual level of physiological variation in *Z. capensis*.

Several studies on TEWL in birds have reported that there are strong differences in this parameter among species and among populations of the same species (Tieleman & Williams 2002; Tieleman *et al*. 2003; Sabat *et al*. 2006). However, there is little evidence about the existence of thermal acclimation in TEWL (Haugen *et al*. 2003) and even less evidence on the variability in the response to thermal acclimatization. We found differences in TEWL for the acclimation in the mesic locality of Santiago; nevertheless, higher values found in cold-acclimated birds might be explained as an indirect effect of a greater metabolic rate. Thus, Williams & Tieleman (2000) suggested that an increase in oxygen consumption causes an increase in breathing frequency, and therefore a greater breathing water loss rate, contributing to an increase in TEWL. This alternative is supported by the correlation between BMR and TEWL in *Z. capensis*. This study suggests that the response to acclimation may vary among physiological traits (see Tieleman *et al*. 2003). Since the variability in TEWL explained by BMR is only 31%, further efforts will be required to determine to what extent the differences found in TEWL among populations and among acclimatized birds are due to skin permeability modifications, and what is relative contribution of breathing water loss and skin water loss to the total water loss by evaporation in *Z. capensis*.

Physiological diversity among individuals, populations and species may be determined by several factors: genetic, development (e.g. irreversible modifications due to environ-mental changes which occur in early stages of development, Piersma & Drent 2003); reversible environment changes (i.e. physiologic flexibility) (Dawson 1984; Swanson & Olmstead 1999; Nilsson 2002) or by a combination of these factors. The reduced BMR and TEWL in *Z. capensis* from the semi-arid environment, coupled with the absence of physiological flexibility in adult birds, suggest that the mechanisms to face little water availability and higher environmental temperatures might have a genetic basis (Broggi *et al*. 2004). Nevertheless, the existence of development plasticity of the energetic character and of evaporative water loss cannot be discarded; in other words in birds from steady environments (e.g. Copiapo) the BMR might be modified in early stages of development but not in the adult stage (Piersma & Drent 2003). Alter-natively, as was proposed by McKechnie (2007), the rapidity of response to thermal acclimation could vary among bird species and populations. Hence, it is possible that the kinetic pattern of flexibility for avian BMR could potentially differ between mesic and semi-arid populations of *Z. capensis*. In this sense, if the time period required for complete acclimation in sparrows from Copiapo is longer than the experimental acclimation time, an underestimation of physiological flexibility could be obtained. Summarizing, in this study we have reported population differences to thermal acclimation in *Z. capensis*. This difference is expressed in higher adjustments in BMR in birds from localities that present high variability in the aridity index. To our knowledge this study is the first evidence for a correspondence between the magnitude of physiological acclimation in BMR and ecological flexibility (seasonal changes) of natural populations in birds.

Acknowledgements

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