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Phenotypic flexibility in the basal metabolic rate of laughing doves: responses to short-term thermal acclimation

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Summary

Many birds exhibit considerable phenotypic flexibility in maintenance energy requirements, and up- or downregulate basal metabolic rate (BMR) over time scales of days to weeks during thermal acclimation. However, the extent to which individual birds can reverse the direction of BMR adjustments over short time scales remains unknown. In this study, we examined metabolic responses to short-term thermal acclimation in laughing doves *Streptopelia senegalensis*. In 30 wild-caught doves (mean body mass=92.6 g) divided into three experimental groups of 10 birds each, initial BMR averaged 0.760±0.036 W. Thereafter, each group was acclimated to one of three acclimation air temperatures (T_{acc}=10, 22 or 35°C) for 21 days, during which time the doves were housed in individual cages. Following the first acclimation period (acclimation I), BMR (W) was significantly lower and was negatively and linearly related to T_{acc} [BMR=0.714–0.005T_{acc}]. Acclimation I BMR varied from 0.546±0.039 W in doves acclimated to T_{acc}=35°C to 0.665±0.058 W at T_{acc}=10°C. A second acclimation period of a further 21 days (acclimation II) revealed that the direction of BMR adjustments could be reversed within individuals, with acclimation II BMR again negatively and linearly related to T_{acc}. The slope of the relationship between BMR and T_{acc} following acclimation II was not significantly different to that following acclimation I. BMR exhibited consistent inter-individual variation, with a low but significant repeatability of 0.113. The within-individual BMR variation of up to 26% that we observed in laughing doves reveals that BMR is a highly flexible trait in this species, and reiterates the need to take phenotypic plasticity into account in comparative analyses of avian energetic parameters.

Key words: acclimation, basal metabolic rate, phenotypic flexibility, thermoregulation, repeatability.

Introduction

In birds, phenotypic flexibility in metabolic power output is an important component of thermoregulatory responses to seasonal environments and accommodating the elevated energy requirements associated with long-distance migration. In many species, adjustments of basal metabolic rate (BMR), summit metabolism (M_{sum}) and/or maximal metabolic rate (MMR) comprise important components of seasonal acclimatization (Liknes et al., 2002; Liknes and Swanson, 1996; Swanson, 1990; Swanson, in press), short-term thermal acclimation (Klaassen et al., 2004; Tieleman et al., 2003b; Williams and Tieleman, 2000), and/or the physiological changes that precede migratory flights between geographically distant breeding and wintering grounds (Battley et al., 2001; Lindström and Klaassen, 2003; Piersma et al., 1995). Collectively, these studies have revealed that BMR is highly flexible in many species, suggesting that phenotypic flexibility may be a general feature of avian metabolic systems (Klaassen et al., 2004). Recent years have seen an increased interest in the adaptive significance of interspecific variation in phenotypic flexibility in metabolic capacity (Tieleman et al., 2003b), and the contribution of phenotypic plasticity to avian metabolic diversity (McKechnie et al., 2006). In this paper, we use the terms phenotypic plasticity, developmental plasticity and phenotypic flexibility following Piersma and Drent (Piersma and Drent, 2003).

Laboratory studies of avian metabolic adjustments associated with short-term thermal acclimation have generally involved comparisons of BMR among experimental groups following acclimation to one of two air temperatures (T_a) (Klaassen et al., 2004; Tieleman et al., 2003b; Williams and Tieleman, 2000). These studies have convincingly demonstrated that several species rapidly adjust the lower limit of metabolic heat production in response to changing thermoregulatory demands, but have not provided any insight into the shapes of BMR reaction norms [(*sensu* Schlichting and

Pigliucci, 1998), i.e. the shape of BMR *vs* acclimation T_a curves]. Moreover, since these experiments involved each experimental bird being acclimated to only one air temperature, they do not reveal the extent to which the direction of these metabolic adjustments is reversible.

In order to be operated on by natural selection, traits must be consistent within individuals (i.e. repeatable) and heritable (Falconer and Mackay, 1996). Although several authors have argued for adaptation in avian BMR (Broggi et al., 2005; Tieleman and Williams, 2000; Tieleman et al., 2003a; Wikelski et al., 2003), the extent to which avian BMR is repeatable within individuals over various time scales has received only limited attention (Bech et al., 1999; Hõrak et al., 2002; Rønning et al., 2005; Tieleman et al., 2003b; Vézina and Williams, 2005). In view of the importance of phenotypic plasticity as a potential contributor to observed interspecific variation in avian BMR (McKechnie et al., 2006; Tieleman et al., 2003b), a better understanding is needed of the interactions between various sources of phenotypic variation in BMR. Specifically, do intraspecific slow–fast BMR continua persist during metabolic adjustments associated with acclimation? In other words, do individuals that exhibit high BMR relative to other members of an experimental population before thermal acclimation maintain their relatively high BMR following acclimation to a new thermal environment? If they do, it would indicate that BMR is potentially a heritable trait that is subject to selection, despite the fact that BMR is variable within individuals and fluctuates over time. There is only one study of which we aware that reported repeatability values for avian BMR during acclimation (Tieleman et al., 2003b).

In this study, we addressed three questions concerning phenotypic flexibility in avian BMR. First, what is the shape of the BMR reaction norm in birds acclimated to more than two air temperatures? Second, to what extent is the direction of BMR adjustments in response to short-term thermal acclimation reversible within individuals? Third, does BMR exhibit significant repeatability during phenotypic adjustments in response to short-term thermal acclimation? We answered these questions using laughing doves *Streptopelia senegalensis*, medium-sized (ca. 95 g) columbids that occur thoughout sub-Saharan Africa and are absent only from true deserts (Hockey et al., 2005).

Materials and methods

Study animals

Forty-five laughing doves *Streptopelia senegalensis* L. were trapped using walk-in traps baited with a wild birdseed mix during July 2005 in Pietermaritzburg, South Africa. Following capture, the doves were individually marked with coloured celluloid split rings and housed in outdoor aviaries (1 m wide×3 m high×3 m long) in the School of Biological and Conservation Sciences at the University of KwaZulu-Natal in Pietermaritzburg. Water, wild birdseed and grit were available *ad libitum*. The first 20 birds caught were weighed upon capture and once a week thereafter. Their body mass M_b was 93.1±6.3 g (mean ± 95% CI) upon capture and decreased by approximately 7.8% during the first week in captivity, before stabilizing. The mean (± s.d.) daily maximum air temperature in Pietermaritzburg while the birds were in the aviaries was 25.2±5.7°C, and the mean daily minimum was 9.9±2.9°C (data from South African Weather Service).

After ca. 28 days, 30 of the doves were transferred to three indoor constant environment rooms (10 doves per room), in which T_a was maintained at 10±2°C, 22±2°C and 35±2°C, respectively. Photoperiods approximately matching the prevailing conditions outdoors were maintained in the rooms. During their time indoors, each dove was housed in an individual cage (40 cm wide×40 cm high×50 cm long), with water, wild birdseed and grit available *ad libitum*. Birds were housed in the cages until the end of experiments.

Oxygen consumption and body temperature measurements

Metabolic rate (MR) was measured indirectly as rate of oxygen consumption ($\dot{V}_{O_2}$) in an open flow-through respirometry system. Each bird was weighed to two decimal places and placed into a 3.96 l clear Perspex respirometry chamber (22 cm high×15 cm long×12 cm wide). Up to five respirometry chambers were placed into a 1 m^3 soundproof temperature-controlled cabinet, with an identical photoperiod to that experienced by the doves in the rooms where they were housed. $\dot{V}_{O_2}$ was measured in each bird either from ca. 17:00 h to 23:30 h, or from ca. 23:30 h to 06:30 h the following morning, with all measurements made during the experimental scotophase.

$\dot{V}_{O_2}$ was measured in an open flow-through system (McKechnie and Lovegrove, 2001), with the fractional O_2 concentration of subsampled air measured using an oxygen analyzer (model S-3A/1, Ametek, Pittsburgh, PA, USA). Before the commencement of measurements, the mass flow meters (Brooks thermal model 5810, Hatfield, PA, USA) that measured the flow rate of excurrent air from each chamber were calibrated to 90% of full scale with a soap bubble flow meter (Baker and Pouchot, 1983). During measurements, dried atmospheric air was drawn through the chambers at 750±190 ml min^{-1}, resulting in <1% O_2 depletion between incurrent and excurrent airflow and 99% equilibrium times of approximately 24 min (Lasiewski et al., 1966). $\dot{V}_{O_2}$ was calculated using equation 3a in Withers (Withers, 1977), and all volumes corrected to STP.

Cloacal body temperature (T_b) was recorded within 30 s of removing each bird from the respirometry system. A fine gauge Cu–Cn thermocouple was inserted approximately 1.5 cm into each bird's cloaca, at which depth a slight withdrawal did not result in a decrease in the T_b reading. If a reliable T_b estimate was not obtained within 30 s of removing each bird, a T_b datum for that bird was not included in the analyses. As a result, sample sizes for T_b, and hence minimum thermal conductance (see below), were smaller than those for BMR and M_b.

Experimental protocol

Determination of the lower critical limit of thermoneutrality

To determine the lower critical limit of thermoneutrality (T_{lc})

and thermoneutral zone (TNZ), and to ensure that all BMR estimates were made at thermoneutral T_a, $\dot{V}_{O_2}$ was measured at 0°C<T_a<32°C. During the $\dot{V}_{O_2}$ measurements, the doves experienced a ramped T_a profile (warm to cold), and spent a minimum of 2 h at 0, 5, 10, 15, 20, 24, 28 and 32°C. The birds experienced no more than four T_a values and a maximum of 12 h in the respirometry chambers on any given night.

The mean of the three lowest $\dot{V}_{O_2}$ measurements (sampling interval=6 min) from the last hour at each T_a was used to calculate resting MR (metabolic rate of a resting, post-absorptive bird at T_a<T_{lc}). The data for each bird were subjectively examined, and a least-squares linear regression model fitted to MR at T_a values below the approximate T_{lc}. The actual T_{lc} was then calculated as the intercept of the linear regression and the minimum MR recorded at any T_a for each individual.

Acclimation I and II experiments

Before the 30 experimental birds were transferred from the outdoor aviaries into the indoor constant environment rooms, T_{lc} was estimated for 15 additional doves as described above. The 30 experimental birds were then randomly split into three groups of 10 individuals each, and each bird's BMR was measured (hereafter referred to as initial BMR) before it was transferred into one of the constant environment rooms (10, 22 or 35°C; Fig. 1). After acclimating to the conditions in the rooms for 21 days, the T_{lc} of the 10 birds at each acclimation air temperature (T_{acc}) was re-determined, and their BMR measured (hereafter referred to as acclimation I BMR). During the T_{lc} re-determination, each bird spent a maximum of two 12-h periods out of the constant environment room where it was housed. BMR was measured in a separate set of measurements at the end of the acclimation I period. Following the measurement of acclimation I BMR, the ten birds in each constant environment room were randomly split into two groups of five birds each, and transferred into the other two rooms. For instance, of the 10 birds acclimated to T_{acc}=10°C, five were moved to the 22°C room, and five were moved to the 35°C room (Fig. 1). The birds were then acclimated to the new thermal conditions before their T_{lc} was re-determined, and their BMR estimated for a third time (hereafter referred to as acclimation II BMR; Fig. 1). Following the acclimation II measurements, the birds were released at the site of capture.

During all three BMR estimates (initial, acclimation I and acclimation II), $\dot{V}_{O_2}$ was measured over a T_a range of 3°C (±1.5°C on either side of the previously determined T_{lc}), in order to ensure that the lowest $\dot{V}_{O_2}$ for each individual did indeed represent basal levels. The birds experienced each T_a for at least 2 h, with the mean of the three lowest consecutive $\dot{V}_{O_2}$ measurements at any one of the three T_a values used to estimate BMR. All BMR estimates were made in birds that could reasonably be considered to be postabsorptive, on the basis of the time elapsed since food was available (4–6 h) (but see Laurila et al., 2003).

Data analysis

All $\dot{V}_{O_2}$ data were subjectively examined, and non-steady state data were excluded from the analyses. Oxygen consumption was converted to metabolic rate (W), using a conversion factor of 20.083 J ml O_2^{-1} (Schmidt-Nielsen, 1990). Assuming that only carbohydrates and lipids were metabolized, the maximum potential error in MR calculated using this approach is 6% (Walsberg and Wolf, 1995) (but see Walsberg and Hoffman, 2005). Minimum wet thermal conductance (C_{min}, mW g^{-1} $°C^{-1}$, i.e. conductance at $T_a \leqslant T_{lc}$ and including evaporative heat loss) was calculated as C_{min}=MR/(T_b–T_a) (Schmidt-Nielsen, 1990). To ensure that estimated conductance was truly minimal, C_{min} for each bird was calculated using $\dot{V}_{O_2}$ and T_b data recorded at T_a slightly (1–3°C) below the T_{lc}.

The M_b-dependence of BMR was assessed by plotting BMR *vs* M_b and fitting a least-squares linear regression model to the data for each of the three BMR measurements (initial, acclimation I and acclimation II) in each of three groups (T_{acc}=10, 22 and 35°C). Since BMR was significantly related to M_b in only one of nine instances (see Results), we used analyses of variance (ANOVA) to compare BMR within and among groups. The experimental design precluded the use of

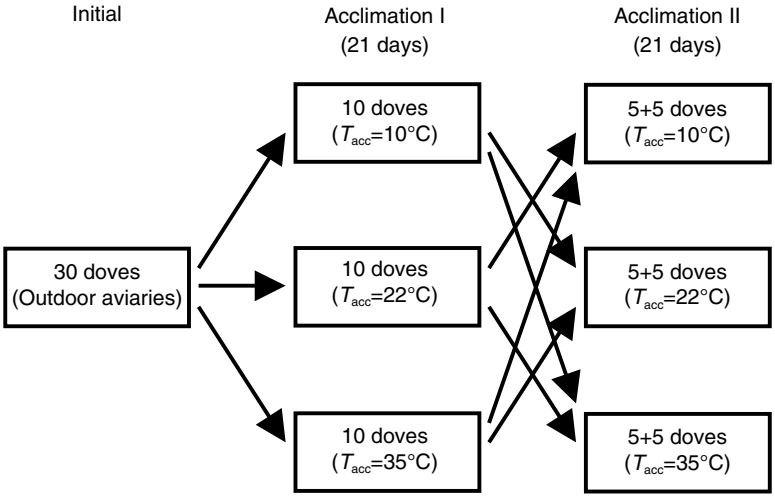

Fig. 1. Summary of experimental design used to acclimate laughing doves *Streptopelia senegalensis* to various acclimation air temperatures (T_{acc}).

a single analysis of the entire data set, since there were three experimental groups for the initial and acclimation I phases, but effectively six for the acclimation II phase, reflecting the fact that each group of 10 birds was split into two groups of five each for the acclimation II phase, and there were thus six sequences of T_{acc}I and T_{acc}II (i.e. 35→10°C, 35→22°C, 22→35°C, 22→10°C, 10→35°C, 10→22°C). Hence, we carried out two analyses. In the first, we tested for experimental effects during the initial and acclimation I phases, using repeated measures ANOVA (RM-ANOVA) with phase (initial or acclimation I) as the independent variable to compare dependent variables (M_b, T_b, C_{min} or BMR) within groups between the two phases, and ANOVA with group (T_{acc}I=10°C, 22°C or 35°C) as the independent variable to compare dependent variables among groups following the acclimation I period. The second analysis we carried out first tested for effects of acclimation history within groups following the acclimation II period. The unbalanced experimental design precluded an ANOVA of the acclimation II data with T_{acc}II and acclimation history as independent variables. To assess whether the acclimation history of an individual affected BMR following acclimation period II, we tested for an effect of acclimation history within each of the three T_{acc}II groups using T_{acc}I as the independent variable. For instance, within the T_{acc}II=10°C group we compared the BMR of the five individuals for which T_{acc}I=35°C to that of the five individuals for which T_{acc}I=22°C. Since we could detect no effect of acclimation history within any of the three T_{acc}II groups, we then compared pooled acclimation II data (i.e. irrespective of the acclimation histories of individuals) to acclimation I data using RM-ANOVA with phase (acclimation I or acclimation II) as the independent variable, and used ANOVA with group (T_{acc}II=10°C, 22°C or 35°C) as the independent variable to compare dependent variables among groups following the acclimation II period. In the case of variables other than BMR, we do not report all non-significant effects. *Post-hoc* Tukey HSD tests for multiple comparisons were used to identify significant differences within and among groups. All analyses were carried out following Zar (Zar, 1999). Unless otherwise stated, values are presented as mean ± 95% confidence interval (CI). When fitting regression models to BMR data, we identified the model that provided the best fit by comparing r^2 values for linear regressions of observed *vs* predicted values among models (Song et al., 1997).

We calculated repeatability (r) for BMR from ANOVA variance components (Lessells and Boag, 1987). To account for the effects of acclimation and T_{acc}, we adopted the approach of Tieleman et al. (Tieleman et al., 2003b), and used the mean squares derived from a one-way ANOVA with BMR as the dependent variable and phase, T_{acc} and individual as fixed variables. The standard error of BMR repeatability was calculated (Becker, 1984). Since the BMR repeatability calculated as described above could potentially have been confounded by the various combinations of T_{acc}I and T_{acc}II experienced by the doves, we also calculated BMR repeatability for each of the six groups of five birds each that experienced a unique combination of T_{acc}I and T_{acc}II.

Results

Body mass

During the initial BMR measurements, M_b=92.6±2.4 g (N=30). Body mass did not change during the acclimation I period (RM-ANOVA, $F_{1,54}$=1.080, P=0.303; Table 1), nor did it vary with T_{acc} following acclimation I (ANOVA, $F_{2,27}$=1.565, P=0.228; Table 1). Following the acclimation II period, M_b did not vary with acclimation history within any of the three T_{acc}II groups (Table 2), nor did pooled data vary among the T_{acc}II groups (ANOVA, $F_{2,27}$=1.594, P=0.222; Table 1).

Body temperature

During the initial measurements, the mean T_b of the three experimental groups was 38.2±0.3°C (N=23). There were significant changes in T_b following acclimation I (RM-ANOVA, $F_{1,44}$=23.19, P<0.005; Table 1), with T_b increasing in the T_{acc}=10°C and 22°C groups (Table 1). Among-group variation in T_b following acclimation I, however, was not significant (ANOVA, $F_{2,24}$=3.277, P=0.055). Following the acclimation II period, T_b did not vary with acclimation history within any of the three T_{acc}II groups (Tables 1, 2), nor did pooled data vary among the T_{acc}II groups (ANOVA, $F_{2,22}$=0.240, P=0.791; Table 1).

Basal metabolic rate

With the exception of the initial BMR of the ten doves acclimated to T_{acc}=10°C, there was no consistent significant relationship between BMR and M_b (Fig. 2), irrespective of whether or not these data were $\log_{10}$-transformed. The initial BMR of the three experimental groups averaged 0.760±0.036 W (N=30). BMR decreased significantly during the acclimation I period in all three groups (RM-ANOVA, T_{acc}I=10°C: $F_{1,18}$=4.662, P=0.045; T_{acc}I=22°C: $F_{1,18}$=19.371, P<0.005; T_{acc}I=35°C: $F_{1,18}$=25.191, P<0.005; Table 1). The reduction in BMR was greatest in the birds acclimated to T_{acc}I=35°C (26.2±8.0%), smallest in the birds acclimated to T_{acc}I=10°C (16.2±5.1%), and intermediate in the birds acclimated to T_{acc}I=22°C (20.3±6.2%) (Fig. 3). Following acclimation I, BMR varied significantly with T_{acc} (ANOVA, $F_{2,27}$=6.540, P=0.005; Table 1), with the BMR of the T_{acc}I=10°C group significantly higher than that of the T_{acc}I=35°C group (Fig. 3). Following the acclimation II period, BMR did not vary with acclimation history within any of the three T_{acc}II groups (Tables 1, 2). The acclimation II phase led to similar among-group variation, with BMR again being negatively and linearly related to T_{acc} (Figs 3, 4). Pooled BMR data varied significantly among the three T_{acc}II groups (ANOVA, $F_{2,27}$=4.528, P=0.020; Table 1), and comparisons of BMR between acclimation I groups and pooled acclimation II groups did not reveal any significant effect of phase (RM-ANOVA, T_{acc}=10°C: $F_{1,18}$=0.010, P=0.922; T_{acc}=22°C:

$F_{1,18}$=0.171, P=0.685; T_{acc}=35°C: $F_{1,18}$=0.135, P=0.718; Table 1). The slope of the relationship between BMR and T_{acc} following acclimation I was statistically indistinguishable from that following acclimation II (Fig. 4). During acclimation II, the magnitude of adjustments in BMR within individuals was negatively related to the change in T_{acc} (Fig. 5). BMR exhibited low but significant repeatability during the course of the experiments, with r=0.113±0.188 (± s.e.m.; $F_{29,89}$=2.268,

Table 1. *Mean body mass, lower critical limit of thermoneutrality, body temperature, minimum wet thermal conductance and basal metabolic rate in laughing doves Streptopelia senegalensis acclimated to various thermal conditions*

			Acclimation II		
	Initial	Acclimation I	[T_{acc}I→T_{acc}II (°C)]		Acclimation II (pooled)
T_{acc}=10°C					
M_b (g)	91.8±3.5 (10)	91.6±5.0 (10)	[22→10] [35→10]	103.9±7.2 (5) 94.4±5.7 (5)	93.6±4.6 (10)
T_{lc} (°C)	29.1±1.4 (15)*	30.0±1.6 (10)	[22→10] [35→10]	34.0±1.5 (5) 32.5±1.0 (5)	33.2±1.0 (10)
T_b (°C)	38.4±0.1 (8)	40.5±1.0 (9)	[22→10] [35→10]	40.2±1.6 (5) 38.2±0.9 (5)	39.2±0.8 (8)
C_{min} (mW g^{-1} °C^{-1})	0.901±0.084 (8)	0.704±0.063 (9)	[22→10] [35→10]	0.806±0.260 (5) 1.043±0.141 (5)	0.925±0.160 (8)
BMR (W)	0.762±0.066 (10)	0.665±0.058 (10)	[22→10] [35→10]	0.660±0.069 (5) 0.662±0.085 (5)	0.661±0.053 (10)
T_{acc}=22°C					
M_b (g)	95.8±5.3 (10)	97.5±4.0 (10)	[10→22] [35→22]	96.3±6.8 (5) 90.1±7.2 (5)	93.6±4.5 (10)
T_{lc} (°C)	29.1±1.4 (15)*	29.8±1.9 (9)	[10→22] [35→22]	30.7±1.7 (5) 32.5±2.6 (5)	31.6±1.6 (10)
T_b (°C)	37.9±0.6 (5)	39.8±0.1 (10)	[10→22] [35→22]	38.8±1.1 (5) 37.4±1.5 (5)	39.0±0.9 (7)
C_{min} (mW g^{-1} °C^{-1})	1.204±0.184 (5)	1.211±0.351 (10)	[10→22] [35→22]	0.891±0.164 (4) 1.043±0.141 (3)	0.857±0.101 (7)
BMR (W)	0.762±0.056 (10)	0.612±0.039 (10)	[10→22] [35→22]	0.626±0.059 (5) 0.575±0.056 (5)	0.600±0.042 (10)
T_{acc}=35°C					
M_b (g)	90.4±4.8 (10)	94.5±4.1 (10)	[10→35] [22→35]	91.0±6.8 (5) 96.3±6.1 (5)	99.2±5.3 (10)
T_{lc} (°C)	29.1±1.4 (15)*	32.4±1.9 (9)	[10→35] [22→35]	31.4±3.9 (5) 33.9±1.6 (5)	32.7±2.1 (10)
T_b (°C)	38.3±0.4 (10)	38.9±1.4 (8)	[10→35] [22→35]	38.2±1.2 (5) 39.1±1.1 (5)	38.8±0.7 (10)
C_{min} (mW g^{-1} °C^{-1})	0.871±0.094 (10)	0.876±0.222 (8)	[10→35] [22→35]	0.778±0.143 (4) 0.930±0.320 (4)	0.854±0.172 (8)
BMR (W)	0.757±0.073 (10)	0.546±0.039 (10)	[10→35] [22→35]	0.566±0.089 (5) 0.549±0.053 (5)	0.557±0.049 (10)

M_b, body mass; T_{lc}, lower critical limit of thermoneutrality; T_b, body temperature; C_{min}, minimum wet thermal conductance; BMR, basal metabolic rate.

Values are means ± 95% CI; sample sizes in parentheses. Initial values are those of unacclimated birds held in outdoor aviaries; Acclimation I values were measured following a 21-day acclimation period; acclimation II values were measured following a further 21-day acclimation period.

Acclimation II data are presented in two ways: for each of the six combinations of acclimation air temperature (T_{acc}) I and II, and pooled according to T_{acc}II. Note that whereas the initial and acclimation I values for each T_{acc} were measured in the same individuals, values for acclimation II were not.

*Values from 15 additional birds not used in main experiment.

Table 2. *Summary of ANOVA results comparing body mass, lower critical limit of thermoneutrality, body temperature, minimum wet thermal conductance and basal metabolic rate among laughing doves Streptopelia senegalensis during the Acclimation II phase*

	T_{acc}=35°C (10→35°C *vs* 22→35°C)		T_{acc}=22°C (10→22°C *vs* 35→22°C)		T_{acc}=10°C (22→35°C *vs* 35→10°C)	
	F	*P*	*F*	*P*	*F*	*P*
M_b (g)	$F_{1,8}$=1.291	0.289	$F_{1,8}$=1.147	0.315	$F_{1,8}$=4.201	0.075
T_{lc} (°C)	$F_{1,8}$=1.434	0.265	$F_{1,8}$=1.249	0.296	$F_{1,8}$=2.864	0.129
T_b (°C)	$F_{1,6}$=0.720	0.429	$F_{1,5}$=0.559	0.488	$F_{1,8}$=2.479	0.154
C_{min} (mW g^{-1} °C^{-1})	$F_{1,6}$=0.360	0.570	$F_{1,5}$=1.890	0.227	$F_{1,8}$=4.275	0.073
BMR (W)	$F_{1,8}$=0.092	0.769	$F_{1,8}$=1.526	0.252	$F_{1,8}$=0.001	0.973

M_b, body mass; T_{lc}, lower critical limit of thermoneutrality; T_b, body temperature; C_{min}, minimum wet thermal conductance; BMR, basal metabolic rate.

For each acclimation air temperature (T_{acc}), values were compared among two groups of birds that experienced different sequences of T_{acc}I and T_{acc}II.

P=0.004). The low overall repeatability did not appear to be affected by the various combinations of T_{acc}I and T_{acc}II experienced by different groups: only in one of the six groups of five birds (22→35°C) was BMR significantly repeatable (*r*=0.286±0.242).

Minimum wet thermal conductance

During the initial measurements, the C_{min} of the doves averaged 1.103±0.133 mW g^{-1} °C^{-1} (*N*=23), and did not vary among the three groups (Table 1). Following the acclimation I period, C_{min} varied with T_{acc} (ANOVA, $F_{2,24}$=4.138, *P*=0.028; Table 1). Following the acclimation II period, C_{min} did not vary with acclimation history within any of the three T_{acc}II groups (Tables 1, 2), nor did pooled data vary among the T_{acc}II groups (ANOVA, $F_{2,22}$=0.278, *P*=0.759; Table 1).

Discussion

Our data reveal that laughing doves exhibit considerable phenotypic flexibility in BMR, and can adjust BMR by up to 26% over a 21-day period. Within individual doves, BMR adjustments involved two components: first, a decrease in BMR following the initial measurements, and second, up- or downregulation of BMR correlated with T_{acc}. The negative relationships between BMR and T_{acc} in laughing doves following both acclimation I and II are consistent with the findings of several workers that the metabolic machinery of birds is upregulated in response to elevated thermoregulatory demands (Klaassen et al., 2004; Tieleman et al., 2003b; West, 1972; Williams and Tieleman, 2000). The magnitude of BMR adjustments in laughing doves, when expressed relative to the T_{acc} gradient, falls within the range observed in other species (Table 3). The birds in our study shifted BMR by 0.8% BMR °C^{-1}, approximately half the magnitude of the adjustments of ca. 1.5% BMR °C^{-1} observed in Hoopoe and Dunn's larks (Tieleman et al., 2003b; Williams and Tieleman, 2000). Our data do not provide a clear picture of the respective contributions of metabolic and insulation adjustments to short-term thermal acclimation, but the observation that individuals acclimated to T_{acc}=10°C exhibited a higher T_b and reduced C_{min} suggests that adjustments in both heat production and heat transfer properties were involved.

Whereas previous studies involved the acclimation of birds to two T_{acc} values, we acclimated laughing doves to three T_{acc} values. Over 10°C≤T_{acc}≤35°C, the BMR reaction norm was approximately linear (Fig. 4). Moreover, BMR adjustments were reversible, with the doves exhibiting similar BMR *vs* T_{acc} curves after acclimation I and II, respectively (Fig. 4). These data reveal that the metabolic adjustments made by laughing doves in response to changes in thermoregulatory demands are reversible over short time scales. Upregulation of BMR may be an important component of improved cold tolerance in many small birds (Swanson, in press), and numerous studies have documented shifts in avian BMR associated with seasonal acclimatization (Liknes et al., 2002; Liknes and Swanson, 1996; Maddocks and Geiser, 2000; O'Conner, 1995; Saarela and Hohtola, 2003; Swanson, 1990; Swanson, 1991; West, 1972). Similarly, many mammals adjust BMR seasonally, with the magnitude, direction and functional significance of metabolic adjustments varying across M_b classes (Lovegrove, 2005). However, the temporal dynamics of seasonal shifts in BMR have not been investigated.

Changes in BMR can result from adjustments in body composition and/or the metabolic intensity of specific tissues (Swanson, in press). In many cases, upregulation of avian BMR associated with premigratory adjustments or enhancements in cold tolerance occurs primarily through changes in the masses of central organs, such as the heart, liver and digestive organs (reviewed in Swanson, in press). The 'energy demand' hypothesis predicts that the masses of the major organs responsible for energy supply are adjusted in response to changes in energy demand, and is supported by data for several species of larks acclimated to warm or cold T_{acc} (Tieleman et al., 2003b; Williams and Tieleman, 2000). However, increases in the oxidative capacity of skeletal muscles are common during cold acclimation/acclimatization, and may contribute to

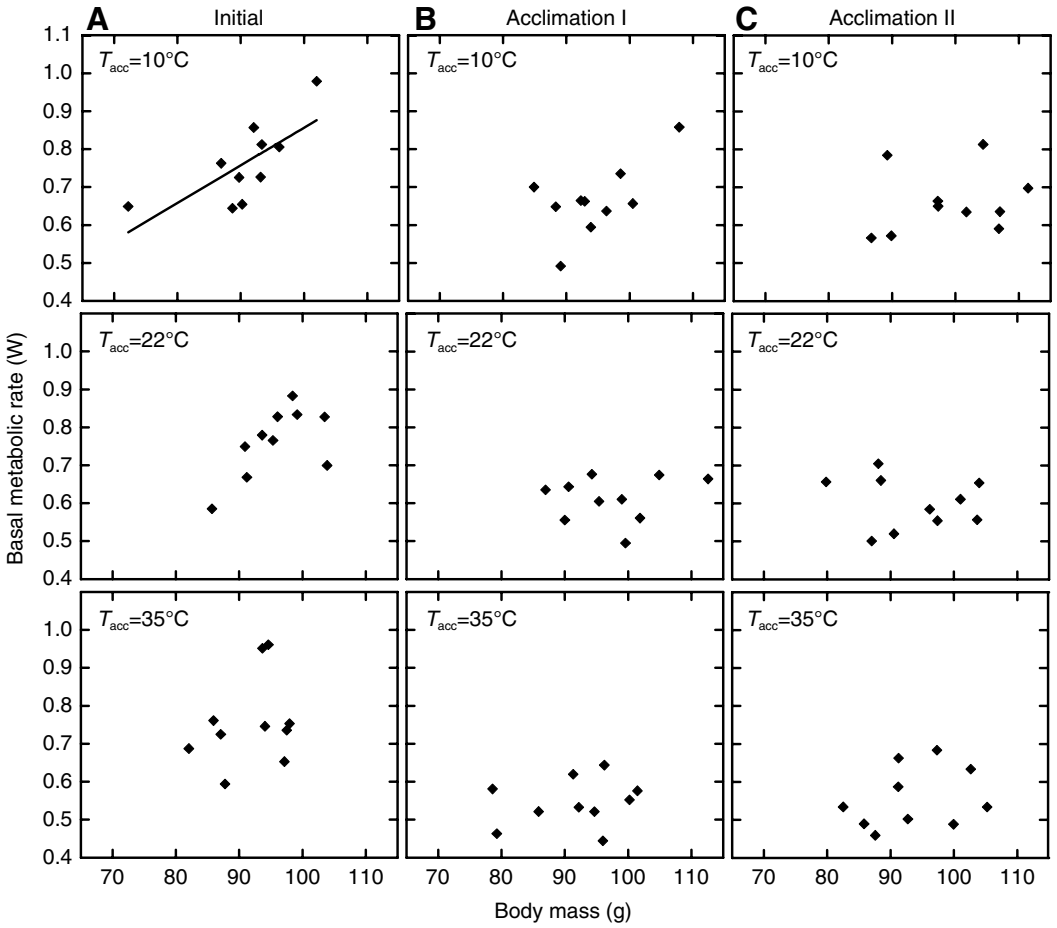

Fig. 2. Basal metabolic rate in laughing doves *Streptopelia senegalensis* was not related to body mass during initial, acclimation I or acclimation II measurements. A linear regression model yielded a significant fit in only one case [initial measurements, acclimation air temperature (T_{acc})=10°C; BMR=0.0099M_b–0.1348; r^2=0.515]. Note that, for each T_{acc}, data for initial (A) and acclimation I measurements (B) were obtained from the same individuals, but from different individuals during acclimation II measurements (C).

increased BMR. For instance, pectoralis muscle in cold-acclimated rock doves *Columba livia* exhibited several ultrastructural changes correlated with enhanced shivering capacity, including reduced fiber cross-sectional area, increased capillary density, and increased mitochondrial density (Mathieu-Costello et al., 1998). In laughing doves, pectoralis muscles comprise 10.9±0.33% of total wet M_b (K.C., A.E.M. and B.G.L, unpublished data). In one of the few studies of the importance of improved shivering thermogenesis *via* enhancements in oxidative capacity on BMR, a significant correlation was observed between the mass of breast muscles and BMR as well as maximum oxygen consumption in house sparrows *Passer domesticus* (Chappell et al., 1999). On an interspecific basis, M_{sum} and BMR are correlated in birds (Rezende et al., 2002).

An unexpected observation in our study was that the BMR

Table 3. *Adjustments in avian BMR associated with short-term thermal acclimation*

	Acclimation		BMR adjustment	
Species	Air temperature (°C)	Period (days)	(% BMR °C^{-1})	Source
Skylark *Alauda arvensis*	15, 35	21	1.3	1
Woodlark *Lullula arborea*	15, 35	21	0.2	1
Hoopoe lark *Alaemon alaudipes*	15, 35	21	0.9	1
	15, 36	21	1.5	2
Dunn's lark *Eremalauda dunni*	15, 35	21	1.5	1
Spike-heeled lark *Chersomanes albofasciata*	15, 35	21	0.6	1
Garden warbler *Sylvia borin*	4, 24	150	0.9	3
Laughing dove *Streptopelia senegalensis*	10, 22, 35	21	0.8	Present study

Note that the BMR adjustments for laughing doves were reported within individuals, whereas those for other species were reported among experimental groups.

[1](Tieleman et al., 2003b); [2](Williams and Tieleman, 2000); [3](Klaassen et al., 2004).

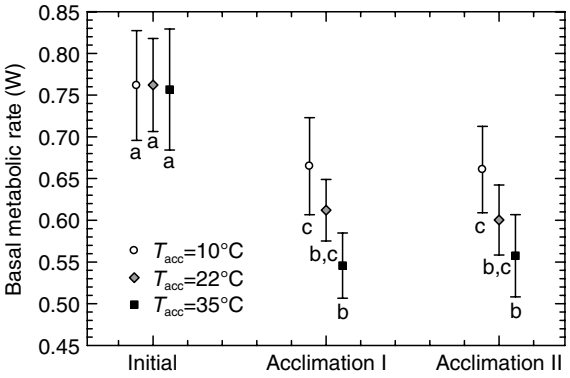

Fig. 3. Basal metabolic rate (BMR) in laughing doves *Streptopelia senegalensis* decreased following initial measurements. Following acclimation I and II, BMR varied with acclimation air temperature (T_{acc}). In each case, BMR was negatively related to T_{acc}. Significant differences are denoted by different lower-case letters: values that are not significantly different from each other share a letter. N=10 individuals for each datum point. Note that, for each T_{acc}, data for initial and acclimation I measurements were obtained from the same individuals, but acclimation II measurements represent different individuals.

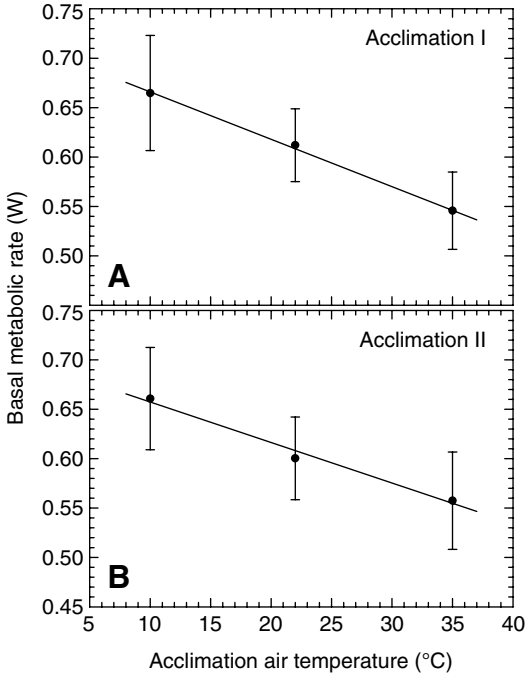

Fig. 4. Following acclimation I (A; BMR=0.714–0.005T_{acc}, r^2=0.325, $F_{1,28}$=13.527, P=0.001) and acclimation II (B; BMR=0.698–0.004T_{acc}, r^2=0.247, $F_{1,28}$=9.208, P=0.005), basal metabolic rate (BMR) in laughing doves *Streptopelia senegalensis* was linearly related to acclimation air temperature (T_{acc}).

of all three experimental groups decreased following the initial BMR measurements. One possible explanation is that the birds were less stressed during the acclimation I and II measurements, having greater familiarity with the respirometry chambers than during the initial measurements. However, in a separate experiment, BMR was repeatedly measured in each individual every 4–5 days following initial measurements, and the differences between initial and acclimated BMR estimates were not significantly different to those reported here (K.C., A.E.M. and B.G.L., unpublished data). An alternative explanation for the initial decreases in BMR, which we consider more likely, concerns the probable contribution of flight muscle maintenance to avian BMR. Since the activity levels of the doves were much lower in the individual cages they were housed in following the initial measurements (which were too small to permit flight) than in the outdoor aviaries, we speculate that the decreases in BMR observed in all three groups reflect reductions in the mass and/or metabolic intensity of their flight muscles. In rock doves, pectoral muscle mass was higher in sedentary birds housed in small cages than in active birds housed in aviaries large enough to permit flight, but the oxidative capacity of pectoral muscles (total and mass-specific cytochrome *c* oxidase activities) was significantly greater in the active birds (Saarela and Hohtola, 2003). Metabolic and thermal responses to seasonal acclimatization occurred independently of metabolic adjustments associated with activity *vs* inactivity (Saarela and Hohtola, 2003).

Several authors have reported significant and high repeatability values for avian BMR (Bech et al., 1999; Hõrak et al., 2002; Rønning et al., 2005; Tieleman et al., 2003b; Vézina and Williams, 2005), suggesting that BMR may indeed be subject to direct selection if evolutionary adjustments in normothermic minimum maintenance metabolism affect inclusive fitness. Although the repeatability we observed for laughing dove BMR during short-term thermal acclimation is lower than most of the values reported in previous studies, it nevertheless reveals that intraspecific slow-fast metabolic continua partially persist during BMR adjustments during thermal acclimation. This finding is consistent with the significant repeatabilities for BMR in three out of five species of larks (Alaudidae) acclimated under laboratory conditions (Tieleman et al., 2003b).

Phenotypic plasticity in avian BMR: implications for comparative analyses

The available data support the view that phenotypic plasticity is a general property of avian metabolic systems (Klaassen et al., 2004). Phenotypic plasticity in avian BMR has far-reaching implications for comparative analyses and the inference of adaptation (McKechnie et al., 2006; Williams and Tieleman, 2000). In the present study, the BMR of laughing doves varied from 78% (acclimation I, T_{acc}=35°C group) to 112% (initial, T_{acc}=22°C group) of the value expected on the basis of McKechnie et al.'s allometric equation for wild-caught birds (McKechnie et al., 2006). Similarly, the BMR of Hoopoe larks varied from 63% to 101% of the predicted value, depending on acclimation state (Williams and Tieleman, 2000). Hence, the conclusions that would be drawn from comparisons of observed and predicted BMR values are strongly dependent

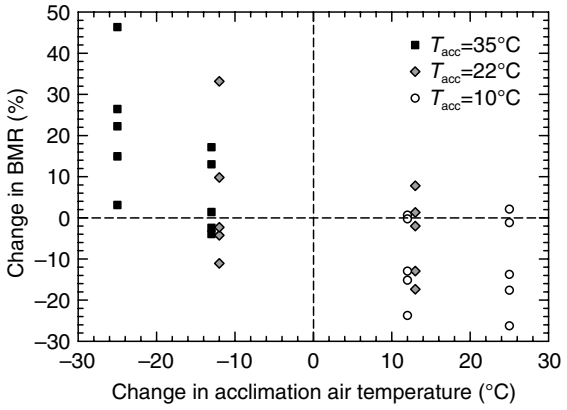

Fig. 5. Acclimation II resulted in adjustments in basal metabolic rate (BMR, W) in laughing doves *Streptopelia senegalensis* that were negatively related to the change in acclimation air temperature (T_{acc}). The relationship between the change in BMR (ΔBMR) within individuals and the change in T_{acc} (ΔT_{acc}) was best described by a cubic regression model $\Delta BMR=-3.5634-0.4394\Delta T_{acc}+0.0148(\Delta T_{acc})^2-0.0004(\Delta T_{acc})^3$ (r^2=0.493, $F_{3,26}$=8.414, P<0.001), where ΔBMR is the change in BMR expressed as a percentage of acclimation I BMR and ΔT_{acc} is the change in T_{acc} between acclimation I and II.

on the thermal conditions preceding acclimation (Williams and Tieleman, 2000).

Adaptation in avian BMR, and possibly other endotherm physiological traits, cannot be reliably inferred from interspecific comparisons unless phenotypic plasticity is carefully controlled for (e.g. Mueller and Diamond, 2001; Wikelski et al., 2003). In studies correlating physiological variation with environmental factors such as temperature and habitat aridity (Lovegrove, 2000; Schleucher and Withers, 2002; Tieleman and Williams, 2000; Williams, 1996), or organismal traits such as diet (McNab, 1986; McNab, 1988; Schleucher and Withers, 2002), variation remaining after M_b and phylogeny are accounted for represents some combination of adaptation and phenotypic plasticity, and cannot be assumed to represent adaptation only. A related issue concerns the ways in which phenotypic plasticity influences the outcomes of statistical procedures for detecting and controlling for phylogenetic non-independence of data. Statistical procedures for detecting phylogenetic signal, most notably the parameters K (Blomberg et al., 2003) and λ (Freckleton et al., 2002; Pagel, 1999), as well as widely used approaches to controlling for phylogenetic effects, namely independent contrasts (Felsenstein, 1985; Garland et al., 1992), PI-ANCOVA (Garland et al., 1993; Garland and Ives, 2000) and generalized least squares (Freckleton et al., 2002; Martins and Hansen, 1997; Pagel, 1994; Pagel, 1999), implicitly assume that the trait value(s) for each tip in a phylogeny is a fixed, taxon-specific value. In the case of avian BMR, the reality is that the datum for each tip can vary substantially depending on acclimation and/or acclimatization prior to metabolic measurements. Liknes and Swanson, for instance, reported seasonal adjustments of ca. 50% between summer and winter in white-breasted nuthatches *Sitta carolinensis* (Liknes and Swanson, 1996), whereas Piersma et al. reported seasonal BMR differences of 110% in captive red knots *Calidris canutus* (Piersma et al., 1995). The magnitudes of such phenotypic adjustments in BMR raise questions about their influence in analyses where the strength of a phylogenetic signal is inferred from tip BMR data and/or phylogenetic non-independence is corrected for by manipulating such data. Although multiple approaches to detecting phylogenetic signals and accounting for phylogeny have been developed in the last two decades, and have been widely employed in comparative analyses of physiological data, phenotypic plasticity in traits such as avian BMR significantly complicates such analyses. Partitioning physiological variation into phylogenetic inertia, adaptation and phenotypic plasticity presents a significant emerging challenge to evolutionary and ecological physiologists.

List of symbols and abbreviations

BMR	basal metabolic rate
C_{min}	minimum wet thermal conductance
M_b	body mass
MMR	maximal metabolic rate
MR	resting metabolic rate
M_{sum}	summit metabolism
STP	standard temperature and pressure
T_a	air temperature
T_{acc}	acclimation air temperature
T_b	body temperature
T_{lc}	lower critical limit of thermoneutrality
TNZ	thermoneutral zone
$\dot{V}_{O_2}$	rate of oxygen consumption

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