Individually variable energy management during egg production is repeatable across breeding attempts

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SUMMARY

It is axiomatic that whole-animal metabolism, measured for example as daily energy expenditure (DEE), plays a central role in determining reproductive success and survival (fitness) in all organisms. Nevertheless, strong evidence for consistent systematic relationships between DEE and either individual traits (age, sex, body size), environmental factors (e.g. food availability, temperature) or 'fitness' traits (e.g. number of offspring, survival) remains far from compelling in birds and mammals. Recently, we suggested that female birds might utilise complex, individually variable energy management strategies to meet the metabolic demands of reproduction, generating a wide spectrum of effects on reproductive DEE, from overcompensation (net decrease in DEE) to additive effects (net increase in DEE). Here we show that this individually variable adjustment or 'plasticity' in energy expenditure associated with egg production is repeatable among individuals between successive breeding attempts in female zebra finches (*Taeniopygia guttata*). Our study highlights the importance (a) of measuring 'plasticity' or change associated with transitions of physiological state (e.g. non-breeding to breeding) based on multiple measurements of the same individual, and (b) of extending consideration of how selection might drive the evolution of phenotypic plasticity *per se* to include physiological and metabolic traits.

Key words: daily energy expenditure, energy management, inter-individual variation, plasticity, egg production, *Taeniopygia guttata*

INTRODUCTION

Whole-animal metabolism, measured for example as daily energy expenditure (DEE), is widely assumed to play a central role in determining reproductive success and survival, i.e. fitness, in all organisms (Carey, 1996; Speakman, 2008). High energy demands might constrain current reproduction because the supply of energy during single breeding attempts is limited or they might generate negative effects on future reproduction (Stearns, 1992). Thus, if parental investment during reproduction is energetically costly then individuals capable of sustaining higher levels of energy expenditure should be better at reproducing, e.g. they may have larger clutch or litter size or rear more offspring (e.g. Meijer et al., 1989; Rogowitz, 1998; Hammond et al., 1994; Tinbergen and Dietz, 1994; Speakman and Krol, 2005). Despite the intuitive logic of these predictions, evidence for consistent systematic relationships between DEE and individual traits (age, sex, body size), environmental factors (e.g. food availability, temperature) or reproductive- or fitness-related traits (e.g. number of offspring, survival) is far from compelling in birds (Williams and Vézina, 2001) (but see Sanz and Tinbergen, 1999; Fyhn et al., 2001) and mammals (Speakman et al., 2003; Speakman, 2008) [except for relationships with temperature (Speakman, 2000; Anderson and Jetz, 2005)]. One reason for this might be the marked, and largely unexplained, inter-individual variation in energy expenditure seen within any population (e.g. Speakman et al., 1994; Berteaux et al., 1996; McKechnie, 2008; Careau et al., 2008).

Recently, we suggested that female birds might utilise complex, individually variable energy management strategies to meet the metabolic demands of egg production (Vézina et al., 2006). At the individual level, energy investment in egg production in female zebra finches *Taeniopygia guttata* [i.e. increased resting metabolic rate, RMR (Nilsson and Raberg, 2001; Vézina and Williams, 2005)] generated a wide spectrum of effects on DEE, from overcompensation (net decrease in DEE) to additive effects [net increase in DEE (Vézina et al., 2006)]. Although all individuals appeared to compensate for the cost of producing eggs *via* behavioural adjustments [decreased locomotor activity (Vézina et al., 2006)] (see also Houston et al., 1995; Williams and Ternan, 1999), this was individually variable. Consequently, net increases in DEE were associated with relatively high reproductive effort (large increase in RMR) and individuals with low reproductive effort (small increase in RMR) were much better at avoiding this cost, and in some cases even overcompensated for the elevated RMR *via* these behavioural adjustments (Vézina et al., 2006). We proposed that this inter-individual variation might help explain why so few studies of free-living birds have found support for positive relationships between energy expenditure and putative correlated ecological or reproductive variables (Williams and Vézina, 2001), and why some studies report contradictory results in different years (e.g. Stevenson and Bryant, 2000). Similarly, this may explain the lack of systematic intraspecific relationships between resting or basal metabolism and reproductive performance reported in many studies in mammals (Hayes et al., 1992; Johnson et al., 2001; Krol et al., 2003; Speakman et al., 2004; Johnston et al., 2007) and birds (Williams and Vézina, 2001) (see also Blackmer et al., 2005). Here we show that the marked inter-individual variation in the adjustment (or 'plasticity') in DEE associated with egg production in female zebra finches is repeatable, i.e. energy management strategies are not only highly variable but are also consistent within individuals over multiple breeding attempts.

MATERIALS AND METHODS

Zebra finches, *Taeniopygia guttata* Vieillot 1817, were maintained in controlled environmental conditions as previously described (Vézina et al., 2006). All birds received a mixed-seed diet (Panicum and white millet, 50:50), water, grit and cuttlefish bone (calcium) *ad libitum*, and egg-laying birds received 6 g of egg food supplement (20.3% protein, 6.6% lipid) daily, which was always completely consumed by the following day. In the present experiment (hereafter termed trial 2) we formed pairs (single-sex and breeding; see below) using a sub-sample of the individual females that we studied previously [(Vézina et al., 2006) hereafter termed trial 1] and measured locomotor activity, food intake and reproductive effort during a second, repeated cycle of egg laying. Repeat measurement of breeding birds in trial 2 occurred 7 months after trial 1. Six males died between the two trials and so six females in trial 2 were paired with different mates. However, this only affected one measured trait: food intake (see Results). Birds were housed in cages (61 cm×46 cm×41 cm) provided with an external nest box (15 cm×14.5 cm×20 cm); for single-sex pairs access to the nest box was blocked with cardboard. During the breeding experiment, nest boxes were checked daily between 10:00 h and 12:00 h, and all new eggs were weighed (to 0.001 g) and numbered. A clutch was considered complete after two consecutive days with no new eggs. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (692B-94), following the guidelines of the Canadian Committee on Animal Care.

Our investigation of DEE adjustments associated with egg production in zebra finches used a repeated measures approach to compare DEE values of 22 females measured as non-breeders in single-sex pairs and at the one-egg stage of laying (sample sizes, non-breeders N=22, one-egg stage N=22). All birds used in the experiment were first paired as single-sex, non-breeding female pairs. Food consumption and locomotor activity (see below) were measured on day 5, 6 and 7 of the single-sex period, and DEE was measured from day 6 to day 7 using the doubly labelled water (DLW) technique (see below). On day 8, all birds were rearranged into breeding pairs and were given access to the nest boxes. Locomotor activity was monitored starting the following day until clutch completion. Food intake data were recorded the first 2 days after pairing (pre-laying) and again during laying beginning the day prior to laying of the first egg and during the following 4 days. All females had their DEE measured at the one-egg stage (i.e. on the day they laid their first egg) with estimates including a complete ovulation and laying cycle (second egg).

We monitored locomotor activity by using a micro-switch system connected to a cage perch as described previously (Williams and Ternan, 1999; Vézina et al., 2006). Food intake was determined by giving the birds 25 g day^{-1} of seeds in an open 946 ml Ziploc^{TM} food container placed on the cage floor and weighing the seeds remaining in the container after 24 h. Williams and Ternan showed that, on average, females eat slightly more food (4.5%) than males and that this sex effect is significant only on the 2 days preceding the first egg laid [P=0.016 and P=0.052, respectively, in their table 1 (Williams and Ternan, 1999)]. Food intake per pair is therefore a good indicator of female food intake in our experimental context, and we report the pair values (g pair^{-1} day^{-1}) as representative of female energy input, as the proportion of seeds eaten by both sexes remains virtually unchanged throughout our experimental protocol (for details, see Vézina et al., 2006).

We measured DEE using the DLW technique (Lifson and McClintock, 1966; Speakman, 1998) as described before (Vézina et al., 2006). This method has been previously validated by comparison to indirect calorimetry in a range of birds (e.g. Bevan et al., 1995; Visser and Shekkermann, 1999; van Tright et al., 2002). On day one, the animals were weighed (±0.01 g) and a known mass of DLW (*ca.* 67.7% ^{18}O, 32.2% ^{2}H) was administered (i.m., 0.4 g 100 g^{-1} body mass). Syringes were weighed before and after administration (±0.0001 g, Sartorius balance) to calculate the mass of DLW injected. Blood samples were taken after 1 h of isotope equilibration to estimate initial isotope enrichment (Krol and Speakman, 1999). Blood samples were immediately heat sealed into 2×50 ml glass capillaries, which were stored at 4°C. Samples were also collected from unlabelled birds to evaluate the background isotope enrichments of ^{2}H and ^{18}O [method C (Speakman and Racey, 1987)]. Animals were recaptured and bled 24 h post-dosing to estimate isotope elimination rates. Capillaries that contained the blood samples were then vacuum distilled (Nagy, 1983), and water from the resulting distillate was used to produce CO_2 and H_2 [methods described in Speakman et al. for CO_2 (Speakman et al., 1990) and in Speakman and Krol for H_2 (Speakman and Krol, 2005)]. CO_2 production was converted into energy utilisation using a conversion factor of 24.03 J ml^{-1} CO_2, derived from the Weir equation (Weir, 1949) for a respiratory quotient of 0.85. The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom mG, Manchester, UK). We ran three high enrichment standards each day alongside the samples and corrected all the raw data to these standards. Isotope enrichment was converted to values of DEE using a single pool model as recommended for this size of animal (Speakman, 1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser and Schekkermann, 1999). We chose the assumption of a fixed evaporation of 25% of the water flux [equation 7.17 in Speakman (Speakman, 1997)] which has been established to minimise error in a range of conditions (Visser and Schekkerman, 1999; van Tright et al., 2002).

Data were analysed using SAS software (version 9.1, 2002–2003; SAS Institute, Cary, NC, USA). We measured multiple traits at multiple times but had a relatively small sample size (N=22 females) so we did not have sufficient power to analyse data in a single comprehensive, multivariate analysis. We focused our analyses on the change in DEE (ΔDEE), and variability in ΔDEE between trials in egg-laying birds (i.e. between pre-laying and the one-egg stage), as a comprehensive within-trial analysis with a larger sample size has been reported previously (Vézina et al., 2006). We first compared differences in mean trait values between trial 1 and trial 2, i.e. a 'time' effect, using repeated measures ANOVA, or ANCOVA with relevant covariates (GLM procedure; see Results). We calculated repeatability for each trait following Lessells and Boag (Lessells and Boag, 1987), using the intraclass correlation coefficient based on variance components derived from a one-way ANOVA. We then analysed correlates of DEE in egg-laying birds during trial 2 only [in order to confirm results previously reported for trial 1 (Vézina et al., 2006)]. Finally, we compared individual variation in ΔDEE between trials to between-trial differences in all measured traits using correlation analysis.

RESULTS
Variation in body mass and reproductive traits
Female body mass was significantly higher in trial 2 in non-breeding (single-sex) birds ($F_{1,21}$=16.97, P<0.01), at pairing ($F_{1,21}$=9.01, P<0.01) and at the one-egg stage of laying ($F_{1,21}$=12.92, P<0.01; Table 1) compared with values from trial 1: by 4.9%, 4.4% and 2.7%,

respectively. Body mass was highest at the one-egg stage but change in body mass did not differ between trials either for the non-breeding to pre-laying stage ($P>0.20$) or for the pre-laying to one-egg stage ($P>0.15$). There was no difference in mean laying interval (time between pairing and first egg, $F_{1,21}=0.70$, $P>0.4$) or mean clutch size ($F_{1,21}=0.06$, $P>0.8$) between trials. Absolute egg mass was 4% larger in trial 2 ($F_{1,21}=19.7$, $P<0.001$; Table 1) but there was no difference in mean egg mass between trials controlling for female body mass ($F_{1,21}=1.60$, $P>0.2$). Female body mass at the one-egg stage, change in mass between the pre-laying and one-egg stage, mean egg mass and clutch size were all repeatable traits (Table 1); however, non-breeding mass and change in mass from the non-breeding to pre-laying stage were not repeatable ($P>0.05$).

Variation in food intake and locomotor activity

Pre-laying food intake was significantly higher in trial 2 than in trial 1, by 0.72 g (23%; $F_{1,17}=8.95$, $P<0.01$). At the one-egg stage there was a significant male effect and male×trial interaction for food intake (both $P<0.025$). For females paired with the same male there was no difference in food intake between trials ($F_{1,15}=0.55$, $P>0.4$). For females paired with different males there was a marginally significant difference in food intake: 4.5 vs 5.7 g day^{-1} in trial 1 and trial 2, respectively ($F_{1,5}=5.38$, $P=0.068$). However, on average for all pairs food intake was only 3.1% higher during trial 2 compared with trial 1 ($F_{1,21}=0.64$, $P>0.4$) and food intake was repeatable between trials (Table 1).

In both trials there was a marked decrease in locomotor activity between the pre-laying and one-egg stage: trial 1, 1385±203 vs 854±89 hops day^{-1} ($F_{1,20}=13.75$, $P<0.01$); trial 2, 1426±221 vs 640±67 hops day^{-1} ($F_{1,20}=14.09$, $P<0.01$). Locomotor activity did not differ among trials during pre-laying ($F_{1,20}=0.02$, $P>0.8$), but activity was lower during trial 2 in laying birds ($F_{1,20}=4.59$, $P=0.05$). For both single-sex pairs and breeding pairs activity during the 24 h DEE measurement period was highly correlated ($R\geq0.76$, $P<0.001$) with activity measured over the whole time period (2–4 days).

Variation in DEE and ΔDEE

Non-breeding DEE, measured in single-sex pairs, was significantly higher (by 10.5%) in trial 2 compared with trial 1 ($F_{1,21}=12.86$, $P<0.01$). However, controlling for body mass, this difference was not significant ($F_{1,25.7}=1.17$, $P>0.2$). Furthermore, there was no difference in either absolute ($F_{1,21}=0.42$, $P>0.5$) or mass-corrected DEE ($F_{1,22}=0.37$, $P>0.5$) between trials at the one-egg stage.

In trial 2, one-egg DEE was positively correlated with food intake ($R_{22}=0.58$, $P<0.01$), and individuals with high one-egg DEE tended to have larger eggs ($R_{22}=0.36$, $P=0.097$) and show a larger increase in body mass between the non-breeding and one-egg stage ($R_{22}=0.37$, $P=0.087$). However, one-egg DEE was independent of locomotor activity, body mass, change in body mass (from non-breeding to laying) and other measures of primary reproductive output (laying interval, clutch size; $P>0.15$ in all cases).

ΔDEE in individual females associated with egg production (calculated as one-egg DEE–non-breeding DEE, where a positive value indicates a net increase in DEE during laying) was not significant for either trial (trial 1, $t_{22}=1.90$, $P>0.05$; or trial 2, $t_{22}=0.56$, $P>0.5$), i.e. on average DEE was not different for non-breeding or one-egg birds (Fig. 1). However, in both trials there was marked individual variation in ΔDEE between the non-breeding and one-egg stages (Fig. 1B). In trial 1, ΔDEE varied between –17.3 and +24.1 kJ day^{-1} (–27.0% to +65.9% relative to non-breeding DEE) and in trial 2 ΔDEE varied between –17.7 and +11.8 kJ day^{-1} (–30.8% to +23.3%). Both one-egg DEE and ΔDEE were repeatable (Table 1; Fig. 1). Finally, the difference in ΔDEE between trials (where negative values indicate greater 'compensation', i.e. lower DEE or smaller increase in DEE relative to non-breeding values) was independent of the difference in all measured traits between trials (body mass, activity, food intake, reproductive effort).

DISCUSSION

In the present study we have shown that not only is absolute DEE repeatable during egg production in female zebra finches, consistent with other studies (for a review, see Nespolo and Franco, 2007), but also the individually variable adjustment or 'plasticity' in energy expenditure associated with egg production is consistent or repeatable. The present study also confirms the key results of our previous study (Vézina et al., 2006), namely that there is no change in mean DEE associated with egg production in female zebra finches, comparing non-breeding birds with birds at the one-egg stage of laying, but that this masks marked, systematic inter-individual variation in the change in DEE which we suggest indicates individually variable energy management 'strategies'. Although it is clear that there are significant energetic costs associated with egg production (e.g. Ward, 1996; Nilsson and Raberg, 2001; Vézina et al., 2006) females appear to use behavioural mechanisms to modulate these energetic costs. However, our study suggests that individual females do this to varying degrees such that some females

Table 1. Primary reproductive effort, body mass, food intake (g pair^{-1} day^{-1}), locomotor activity and daily energy expenditure during egg-laying, and repeatability estimates for these traits

Parameter	Trial 1	Trial 2	Difference	Repeatability
Laying interval (days)	7.4±2.9	6.7±3.1	n.s.	n.s.
Clutch size	5.7±1.8	5.8±1.4	n.s.	0.412*
Mean egg mass (g)	1.033±0.083	1.076±0.091	$P<0.001$	0.762***
One-egg body mass (g)	15.0±0.9	15.4±0.7	$P<0.01$	0.675***
ΔBody mass (g)	1.36±0.62	1.16±0.46	n.s.	0.422*
Food intake (g pair^{-1} day^{-1})	5.40±1.2	5.57±0.75	$P=0.05$	0.422*
Activity (hops day^{-1})	854±412	640±309	$P=0.05$	0.797***
Non-breeding DEE (kJ day^{-1})	51.3±6.5	56.7±5.0	$P<0.01$	n.s.
Laying DEE (kJ day^{-1})	55.0±6.3	55.9±7.7	n.s.	0.569**
ΔDEE (kJ day^{-1})	3.6±9.0	−0.79±6.32	$P<0.01$	0.475**

Measurements were obtained from $N=22$ female zebra finches during two successive breeding attempts. Repeatability estimates were made following Lessells and Boag (Lessells and Boag, 1987).
Change in body mass was calculated as the pre-laying mass minus the laying mass. Change in DEE (daily energy expenditure) was calculated as one-egg DEE minus non-breeding DEE.
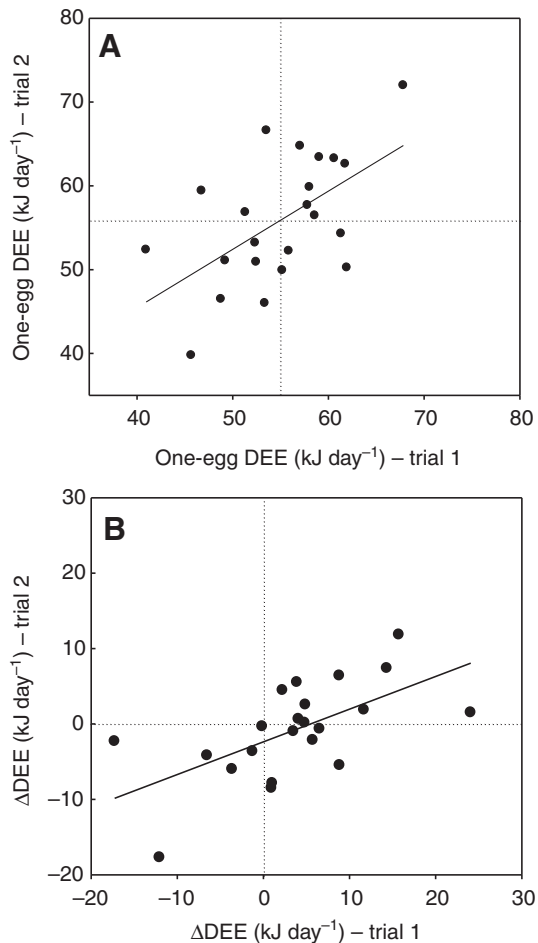Values are means ± s.d.; *$P<0.05$, **$P<0.01$, ***$P<0.001$; n.s., not significant.

Fig. 1. Repeatability of (A) absolute daily energy expenditure (DEE) at the one-egg stage, and (B) change in DEE (ΔDEE) between the non-breeding and one-egg stage in female zebra finches measured in two successive breeding trials.

'overcompensate' for these changes with a decrease in DEE during egg production whereas other females incur additive costs with a net increase in DEE during egg production.

So far we have been unable to resolve the cause of the marked inter-individual variation in DEE or changes in DEE associated with egg production, and this is likely to prove difficult given the potential for (a) behavioural adjustments allowing reallocation of energy among different activities (e.g. Williams and Ternan, 1999; Speakman et al., 2001; Husak, 2006); (b) intrinsic physiological adjustments such as organ remodelling (Vézina and Williams, 2003; Speakman, 2008) or reallocation of energy away from other physiological systems (e.g. Roberts et al., 2004; French et al., 2007); and (c) effects of extrinsic factors such as ecological or social context (Speakman et al., 2003). In the present study the only trait that was strongly correlated with DEE was food intake (see also Vézina et al., 2006). In our opinion this is probably an effect, rather than a cause, of higher energy expenditure, i.e. birds have to increase dietary intake to meet the higher DEE (although it is possible that higher processing costs associated with increased food intake might increase basal metabolic rate (BMR) (Nilsson, 2002) which might in turn contribute to increased DEE (but see Williams and Vézina). We previously reported (Vézina et al, 2006) that females with the highest DEE at the one-egg stage produced larger clutches and

suggested that these females might benefit in terms of reproductive investment despite the 'additive' nature of reproductive energy costs. We could not confirm the relationship between DEE and clutch size in the present study, although there was some evidence to support the idea that individuals with the highest one-egg DEE obtain benefits in terms of reproductive output: there was a trend for DEE to be associated with a larger egg size and a larger change in body mass, perhaps reflecting a higher mass of developing reproductive organs (Vézina et al., 2006). By definition, repeatability is typically calculated using repeat measurements of individuals under similar conditions, as in this experiment, and if DEE is mainly set by extrinsic factors such as food supply (which was constant and *ad libitum* in our experiment) then this might overestimate repeatability related to intrinsic factors (Speakman, 2000; Speakman et al., 2003). Indeed, we found that several components of individual reproductive investment were repeatable including body mass, egg size and clutch size. Although there was minor variation in ΔDEE between breeding attempts this variation itself was not explained by any of the other measured traits (e.g. body mass, egg size, clutch size) or by differences in these traits between the two breeding attempts. Determining the extent to which the repeatability of ΔDEE is robust under varying breeding conditions would obviously be a priority for future studies.

Our study adds to the growing evidence for the repeatability of different measures of energy expenditure, including BMR (Bech et al., 1999; Labocha et al., 2004; Rønning et al., 2005) (but see Russell and Chappel, 2007), RMR (Fournier and Thomas, 1999; Vézina and Williams, 2005) and DEE (Potti et al., 1999; Nespolo and Franco, 2007) (but see Berteaux et al., 1996). However, we have also shown that it is important to be able to measure 'plasticity' or change in energy expenditure associated with transitions of physiological state (e.g. non-breeding to breeding) based on multiple measurements of the same individual (see McKechnie, 2008). An increasing number of ecological and evolutionary studies have highlighted the importance of considering how selection might drive the evolution of phenotypic plasticity *per se* not just absolute trait values (e.g. Piglucci, 2005; Brommer et al., 2008); our study shows that it will be important to extend this consideration of plasticity to physiological, endocrinological and metabolic traits (see also Williams, 2008; Careau et al., 2008).

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
DEE	daily energy expenditure
DLW	doubly labelled water
RMR	resting metabolic rate
ΔDEE	change in DEE

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