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 s .3daTsl 1-ing attempts is fautted or they might g sUin2y

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

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%,

‘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

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