Integrative and Comparative Biology

Integrative and Comparative Biology, volume 54, number 4, pp. 539–554
doi:10.1093/icb/icu095

Society for Integrative and Comparative Biology

SYMPOSIUM

Early-Developmental Stress, Repeatability, and Canalization in a Suite of Physiological and Behavioral Traits in Female Zebra Finches

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From the symposium "Stress, Condition and Ornamentation" presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2014 at Austin, Texas.

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Synopsis Adaptive developmental plasticity allows individuals experiencing poor environmental conditions in early life to adjust their life-history strategy in order to prioritize short-term fitness benefits and maximize reproductive output in challenging environments. Much research has been conducted to test whether such adoption of a "faster" life-history strategy is accompanied by concordant changes in behavior and physiology, with mixed results. As research in this field has focused on comparison of mean-level responses of treatment groups, few studies include repeated measures of response variables and the effect that developmental stress may have on repeatability *per se*. We investigated how early-developmental stress affects the mean expression of (and repeatability in) a variety of behavioral and physiological traits in female zebra finches. We predicted that: (1) individuals subjected to nutritional restriction in the nestling phase would have higher feeding and activity rates, with associated increases in hematocrit and basal metabolic rates (BMRs), (2) nutritional restriction in early life would alter adults' stress-induced corticosterone level, and (3) developmental stress would, respectively, influence the amount of among-individual and within-individual variation in behavioral and physiological traits, hence affecting the repeatability of these traits. In comparison to control females, stressed females did not differ in activity rate or stress-induced corticosterone level, but they did have higher levels of feeding, hematocrit, and BMR. Among-individual variance and repeatability were generally higher in stressed females than in controls. Finally, we found that developmental dietary restriction significantly reduced the amount of within-individual variance both in activity rate in the novel environment and in stress-induced corticosterone level. Our results not only confirm previous findings on the effect of early-developmental stress on BMR, but also extend its effect to feeding rate and hematocrit, suggesting that developmental plasticity in these traits is ontogenetically linked. Early-developmental stress may disable particular genetic canalizing processes, which would release cryptic genetic variation and explain why repeatability and among-individual variance were generally higher in the stressed groups than in controls. For activity rate in the novel environment and with stress-induced corticosterone level, however, early-developmental stress significantly reduced within-individual variance, which may be a consequence of increased canalization of these traits at the micro-environmental level.

Introduction

Early-developmental experiences in vertebrates have profound effects on life-history strategies expressed in later life (West-Eberhard 2003). These effects potentially allow for adaptive developmental plasticity, which enables animals to adopt a life-history strategy that maximizes their fitness in a changing world (Monaghan 2008). Such fine-tuning of life-history strategies should be accompanied by concordant changes in behavior and physiology, leading to defined suites of metabolic, behavioral, and life-history traits (Réale et al. 2010). Developing offspring can detect environmental signals that allow them to adaptively alter their phenotype to better suit the predicted environmental factors they are likely to encounter as an adult (Dantzer et al. 2013). The occurrence of such developmental plasticity across broad taxonomic groups suggests that there is selection for

Advanced Access publication June 25, 2014

ontogenetic processes that maintain developmental plasticity (Monaghan 2008; Moczek et al. 2011).

Across taxa there is strong evidence that the mediators for observed environmental effects are likely to be steroid hormones, both on brain development (Brown and Spencer 2013) and cognitive function (Buchanan et al. 2013). Trade-offs between the functional importance of different cognitive traits may mediate investment in different brain nuclei (e.g., Sewall et al. 2013), and this may underlie the development of different behavioral types. Steroid hormones can have multiple effects on brain and somatic development that then affect a suite of behavioral traits through adulthood (Adkins-Regan 2005; Spencer et al. 2009; Stamps and Groothuis 2010).

In recent years, a body of studies has identified many long-term consequences that early-developmental conditions (Brown and Spencer 2013; Buchanan et al. 2013) or compensatory growth (Metcalfe and Monaghan 2001) can have for adults' life-history strategies, physiology, and behavior. In the black-legged kittiwake (*Rissa tridactyla*), supplemental feeding in early life advances the age of first reproduction, with considerable implication for life-history trade-offs (Vincenzi et al. 2013). In song sparrows (*Melospiza melodia*), early nutritional restriction or exposure to corticosterone resulted in a higher basal metabolic rate (BMR) in adulthood, but only in females (Schmidt et al. 2012). In Japanese quail (*Coturnix coturnix japonica*), exposure to corticosterone in early development affects long-term basal antioxidant defenses both in temporal and in tissue-specific manners (Marasco et al. 2013) and results in increased activity and exploration in a novel environment (Zimmer et al. 2013). In zebra finches (*Taeniopygia guttata*), nestlings' nutritional restriction *per se* did not affect exploratory behavior in later life, but individual variation in compensatory growth rate was negatively correlated with activity and exploratory behavior (Krause and Naguib 2011). In the same species, however, the rate of compensatory growth appears to have no effect on baseline corticosterone level (Honarmand et al. 2010).

Phenotypic consequences of early-developmental stress are usually examined by comparing groups of individuals subjected to different treatments (e.g., testing for differences in the average level of a trait in control versus stressed individuals). Although group-comparisons have greatly aided our understanding of developmental consequences, their narrow focus on the mean attributes of groups has prevented identification and understanding of responses at the individual level and, consequently, has led to a neglect of the considerable value of within-group analyses. Over the past approximately 30 years, behavioral ecologists, endocrinologists, and evolutionary physiologists have increasingly realized the importance of studying individual variation as the substrate on which selection operates (Bennett 1987; Hayes and Jenkins 1997; Réale et al. 2007; Williams 2008). A prerequisite to determine the evolutionary significance of any trait is to quantify its repeatability (R) as the proportion of the total phenotypic variance (V_P) due to variance among individuals (V_I), as opposed to variance within an individual (V_e; Falconer and Mackay 1996). Yet, surprisingly few studies on developmental stress include repeated measures and a quantification of the R in the traits considered, let alone an evaluation of the effect of developmental stress on R *per se*.

Since R is a variance ratio, it can mask some interesting phenomena occurring at the among-individual and within-individual levels. Distinguishing between these sources of variance is informative in its own right as V_I indicates the degree to which individuals differ in their mean expression of a trait and V_e indicates the degree to which repeated observations differ from individuals means (Dingemanse and Dochtermann 2013). Variation among individuals (V_I) is generated by genetic and environmental factors that influence the phenotype permanently (or at least throughout the study). Variation within individuals (V_e) is generated by measurement error, temporal changes in gene expression, or because an organism is sensitive to changes in its micro-environment (i.e., within-individual plasticity toward any stimulus that is statistically unaccounted). Thus, any comparison of R between groups should be accompanied by an analysis of the relative contributions of V_I and V_e in the traits considered (Jenkins 2011).

An emphasis on R, V_I, and V_e connects the study of developmental stress with the key quantitative-genetics concepts of phenotypic plasticity and canalization (Debat and David 2001). Environmental and genetic canalization describe any mechanism, structure, or process, adaptive or not, that will reduce the phenotype's sensitivity to environmental and genetic perturbations (Flatt 2005). Environmental variance can be partitioned into a *macro*-environmental component (i.e., factors shared by many individuals of a given population or treatment, e.g., temperature and availability of food) and a *micro*-environmental component (factors specific to a given individual within a given population or treatment). When studying the effects of early-developmental stress, one usually chooses to manipulate some aspects of the macro-environment. By definition, a macro-environmentally

canalized trait should not be influenced by early-developmental stress (whereas a developmentally plastic trait should). It is unknown, however, whether early environmental stress can reduce the sensitivity of individuals to micro-environmental variation later in their life, which would be observable as a lower V_e in the treatment versus control groups. Early environmental stress could also affect V_I in genetically canalized traits. Indeed, canalized traits show little variation despite an underlying "cryptic genetic variation," which under some circumstance can be released by some decanalization events (Flatt 2005). It may be possible that developmental stress renders canalizing mechanisms nonfunctional, which could lead to higher V_I in the treatment versus control groups.

Our first objective was to test the effect of early-developmental stress on a suite of behavioral traits, including activity and feeding rates in novel and familiar environments, physiological indices of condition such as hematocrit and stress-induced corticosterone level (the principle avian stress hormone), and measurements of metabolic rate (MR) under three different conditions. Second, we sought to test the effect of early-developmental stress on the R of these traits, since R is a useful estimate of the reliability of multiple measurements on the same individual and in some circumstance may define the upper limit of heritability (h^2; Falconer and Mackay 1996, but see Dohm 2002). Third, we estimated the effect of early-developmental stress on V_P, V_I, and V_e, as differences in these components will further inform us as to whether developmental stress acts as a decanalizing agent and/or affects the sensitivity of individuals to micro-environmental variation upon reaching adulthood.

Materials and methods

Animals

In August 2011, 40 breeding pairs of zebra finches were moved into assigned cages ($118 \times 50 \times 50$ cm; 1 pair per cage) under a photoperiod of 14L:10D. All pairs hatched at least one brood by April 2012. Pairs were provided with a nest box and nesting material and their nesting activity was monitored daily. During the period from oviposition through day 5 post-hatch, pairs were given an *ad libitum* diet of seed (Golden Cob finch mix), egg protein, and fresh greens. On completion of the clutch, each pair was randomly assigned to one of two treatment groups: (1) control pairs that received seed *ad libitum* throughout breeding or (2) food-restricted pairs that received a limited amount of food daily, which approximated their daily requirements, mixed in a 3:1 ratio by volume of milled rice husk:volume of seed (Buchanan et al. 2004). These treatment groups were maintained on these diets until day 30 post-hatch, at which time all pairs received *ad libitum* seed and fresh greens daily. All offspring stayed within their sibling groups and within visual and acoustic contact of their parents until day 60 post-hatch, at which time they were moved to another cage ($118 \times 50 \times 50$ cm) and housed with individuals of the same sex from other nests. At approximately day 100 days post-hatch the birds were moved to large outdoor flight cages ($2\,m \times 5\,m \times 3\,m$) with females and males housed in separate cages. Body mass was recorded daily during dietary restriction (post-hatch days 5–30) and every 10 days until post-hatch day 70. The restricted diet reduces the growth rates of nestlings below that of the control group (Spencer et al. 2003), such that variation across the treatment groups broadly resembles the natural variation in the asymptotic mass of fledged wild zebra finches (Mainwaring et al. 2010). All females raised under food-restricted diets are termed "stressed females" and those raised on *ad libitum* diets are referred to as "control females." Shortly before behavioral and physiological testing, the birds were moved back indoors into cages ($118 \times 50 \times 50$ cm), housed separately by sex and held on an *ad libitum* diet. Behavioral and physiological testing was initiated when females were between 455 and 762 days old (mean = 570 days). Females were housed four per cage (dimensions: $118 \times 50 \times 50$ cm; two control and two treatment) at 22°C on a 10-h daylight schedule. A 20-min dimming period occurred from 05:54 to 06:16 (lights on) and between 15:54 and 16:16 (lights off).

Protocol overview

We took four sets of repeated measures (first set: August 20–26, 2013; second set: September 7–14, 2013; third set: November 11–16, 2013; and fourth set: December 10–18, 2013), during which all individuals were measured in batches of 4. For a given batch of four individuals, we completed all behavioral and physiological measures within 50 h (except for the set of fourth measurements in which only behavioral measures were taken; see below). On the first day, birds were moved from their home cage and introduced into a novel cage at approximately 12:00 (median = 12:02; range: 11:58–12:07), in which we monitored locomotor and feeding activity for the following 28 h. At approximately 16:00 on day 2, birds were taken out of the activity cages,

individually placed in holding bags, and transported to another building. Birds were sequentially introduced into an opaque (dark) metabolic chamber at a median time of 16:21 (range: 16:15–16:32) and O_2-consumption was continuously monitored over the following 16 h. At approximately 08:30, birds were transferred into holding cages with access to food and water. At approximately 10:30 (range: 10:29–10:51), we placed each bird in a separate cloth holding bag and left it there for 30 min after which we took a blood sample (median time = 33 min; range: 29–36 min). Immediately after the blood sample, birds were placed in clear metabolic chambers and MR was measured for an additional 3 h under illumination, starting at a median time of 11:10 (range: 11:07–11:28). We wanted to measure MR in the middle of the birds' active phase as it might reflect behavioral adaptive strategies within the population better than MRs measured during the inactive phase (e.g., Careau et al. 2011). Birds were returned to their home cage at 16:00 on day 3.

Locomotor and feeding activity

We monitored locomotor and feeding activity over 28 h in one of two rooms with four cages placed next to each other. The cages used were of a different size (dimensions: 33 × 44 × 47 cm) than the home cages. Moreover, in home cages seeds and water are available in dishes, but in the activity cages these were placed in feeders. We monitored activity using dual-element passive infra-red (PIR) movement detectors (purchased at Jaycar; Cat. No. LA-5044) mounted on top of the individual cages. These detectors are designed to detect movements within a 110° convex honey comb by detecting changes in infra-red radiation levels caused by an endotherm moving within the monitored field. To prevent cross-detection by the movement sensors, we placed cardboard partitions between adjacent cages. Hence, the four birds for a given batch had no visual contact, but could hear each other. Movement triggered a voltage pulse to a "wald test LED" that was monitored via an A-to-D converter. Each cage had a single feeder with a slotted cover, with an infra-red emitter placed on one side of the slot and an infra-red detector on the other. The infra-red beam was broken when the bird's head entered the feeder to get seed, which interrupted the voltage output from the infra-red circuit. In this way, the start and duration of each bout of feeding were detected. Analog signals from both the activity and the feeding detectors were digitized using a UI-2 A-to-D converter (Sable Systems) and stored on a laptop every second

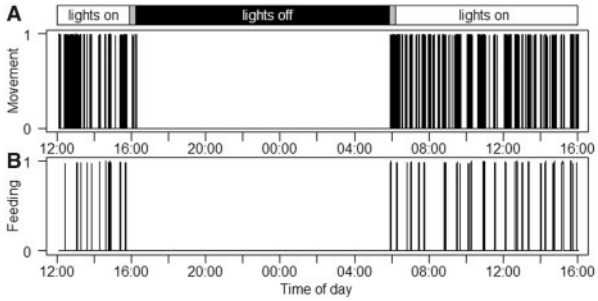

Fig. 1 Representative example of a continuous behavioral recording for a female zebra finch, indicating absence (=0) or presence (=1) of (**A**) locomotor activity and (**B**) feeding over 28 h of monitoring. Lighting status is indicated above (white = light on; gray = dimming period; and black = lights off).

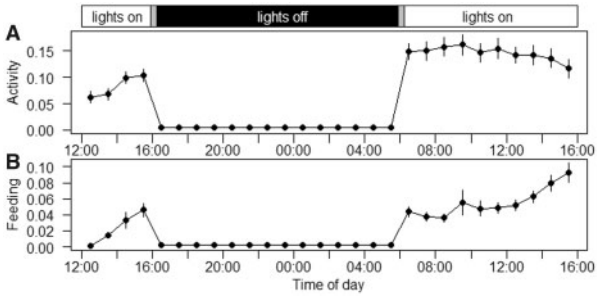

Fig. 2 Average (±se) levels of (**A**) locomotor activity and (**B**) feeding over 28 h of monitoring. Lighting status is indicated above (white = light on; gray = dimming period; and black = lights off).

using Warthog Systems LabHelper (M. A. Chappell, University of California Riverside, Riverside, CA, USA). We transformed the voltage values for recordings of activity and feeding such that the signal was equal to 1 V when movement and feeding occurred and 0 V when these activities were absent (Fig. 1).

For each hour interval, we calculated the average voltage of the activity and feeding recordings, which gives the proportion of time spent active and/or feeding. For example, if a bird spent 25% of the time active over an hour, then average of the 3600 samples would be 0.25 V (2700 and 900 samples of 0 and 1 V, respectively). On average, activity and feeding were initially very low and gradually increased over the first 4 h of recording (Fig. 2), which is a typical behavioral response to exposure to a novel environment (Montiglio et al. 2010). No activity or feeding occurred while lights were off. The activity and feeding patterns over the 10 h of recording during the second day (Fig. 2) are assumed to be indicative of behavior in a familiar environment. Due to technical problems with lighting controls, the first set of measurements was made under

constant daylight; thus, only data from the first 4 h were used for this group. We thus conducted a fourth set of measurements in which only activity and feeding behaviors were measured.

Metabolic rate

We measured MR using a computerized open-circuit respirometry system that allowed us to continuously monitor four chambers simultaneously. For a given metabolic run, four individuals were weighed on a digital balance (±0.01 g; Model OHAUS AV413C) and then placed individually in metabolic chambers fashioned from 1–l paint tins fitted with inlet and outlet ports and a perch. Metabolic chambers were placed in a constant-temperature cabinet regulated at 32°C, which lies within the thermoneutral zone for zebra finches (Calder 1964). A manifold and four mass-flowmeters (Mykrolis, model FC-2902V-T) provided a constant flow of 300 mL min^{-1} of dry, CO_2-free air to each chamber. Excurrent air from each chamber, along with inlet air from a parallel circuit, was sequentially sampled via two computer-controlled multiplexers (Sable Systems models v2.0 and TR-RM4). A 100 mL min^{-1} sub-sample of inlet air or chamber outflow was aspirated from the multiplexer and pulled through Drierite and soda lime before entering two 2-channel oxygen analyzers (Model Oxzilla; Sable Systems International, Henderson, NV, USA). This configuration allowed us to sequentially sample oxygen content of inlet and outlet air from each of the four chambers for 5 and 25 min, respectively, throughout the measurement period. We used Warthog Systems LabHelper software to control the multiplexer outputs and recorded the O_2-concentration and temperature of the chamber at 1-s intervals. Files were transferred to Sable Systems Expedata software and, after correcting the metabolic data for drift between consecutive baseline measures, we calculated individual O_2-consumption according to equation (4a) of Withers (1977).

In endotherms, BMR is defined as the lowest level of energy expenditure expressed by an (1) adult that is: (2) non-reproductive, (3) euthermic, (4) resting, (5) post-absorptive (6) at thermal neutral temperatures, and (7) measured during the resting phase of its daily cycle (McNab 1997). BMR was calculated from the lowest continuous oxygen consumption rate measured over a 5-min interval during the 16-h overnight respirometry run. All birds were probably post-absorptive at the time BMR was measured, which occurred after midnight 75% of the time and always at least 4 h into the run (i.e., after 20:00; see Fig. 3). Daytime resting metabolic rate (RMR) was calculated from the lowest 5-min interval of MR measured over 5 continuous minutes during the 3-h daytime respirometry run. Personal observations indicate that birds were inactive in chambers during these determinations. As the birds were allowed to feed (from 08:30 to 10:30) before daytime RMR measurements and were exposed to daytime lighting, our daytime RMR measures violate BMR criteria #5 and #7 and probably include some additional stress caused by the prior blood sampling. In addition to measures of BMR and RMR based on standard criteria (see above), we also extracted another feature from the overnight metabolic measurements that is potentially relevant. We extracted the "morning MR," which corresponds to the average MR from approximately 06:00 to 08:00 (Fig. 3). Morning MR meets all criteria for BMR except #7 and thus represents the endogenous MR just after the bird has transitioned into the active phase of its daily cycle. The timing of this transition did not seem to differ among individuals (see Supplementary Fig. S2).

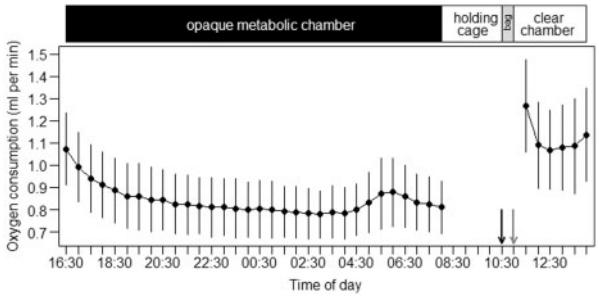

Fig. 3 Average (±SD) levels of MR over 16 h of monitoring overnight in opaque metabolic chambers and 3 h of daytime monitoring in clear metabolic chambers. Lighting status is indicated above (white = light on; gray = in a holding bag; and black = lights off). Black and gray arrows indicate time of disturbance and blood sample, respectively.

Body mass was measured at approximately 16:30 and 08:30 (before and after the overnight metabolic run) and the average of the two was used as a covariate in the subsequent analyses of MR extracted from the overnight respirometry curve. Body mass was also measured after the daytime metabolic run (at ~14:30) and this measure was used as a covariate in the subsequent analyses of daytime RMR.

Blood samples and hematocrit

After approximately 30 min of restraint (time elapsed from capture by hand in the holding cage until removal from the holding bag), blood samples were collected by venipuncture of a brachial vein into

heparinized capillary tubes (Fisher; Cat. No. 22-362-566). Hematocrit was measured using a micro-hematocrit reader (Hawksley; Cat. No. 01502-00). We collected two complete capillary tubes of blood per bird in 49 out of 56 of the sampling events. The hematocrit readings from the two capillary tubes were highly correlated ($r^2 = 0.94$; Supplementary Fig. S1); we thus calculated the average of the two capillary tubes.

Hormone assays

Stressed-induced corticosterone level was measured in triplicate from plasma samples using an Enzo Enzyme Immunoassay kit (Enzo Life Sciences; Cat. No. ADI 900-097). Samples were initially spiked with approximately 1 pg of tritiated corticosterone (1,2,6,7-^{3}H; Perkin Elmer, Australia) prior to steroid extraction to determine percentage recovery. After extraction in dichloromethane, samples were dried under nitrogen and then reconstituted in the manufacturer's buffer solution (1:30 ratio). Hormone values were adjusted for individual sample recovery, which averaged $86.2 \pm 3.4\%$. Reported hormone values are based on the average of the two closest of the triplicate values, except in cases where these had greater than 10% variation. In such instances, remaining plasma for these samples was re-analyzed in an additional assay. Intra-assay variation using triplicate standards and samples was 6.1% and inter-assay variation, assessed using a quail plasma pool, was 9.7%. Sampling of blood occurred at an average ($\pm$SD) of 32.2 ± 1.59 min (range: 29–36 min) after initial disturbance in their holding room. Deviation from the 30-min period did not influence stress-induced corticosterone level ($F_{1,41} = 0.24$, $P = 0.63$) and this variable was left out of the analysis.

Statistical analysis

Analyses were conducted in ASReml-R (Butler et al. 2009). We first used random regression models (Henderson 1982) to test for the effect of dietary restriction on growth rate and during three different phases of the development: the first 10 days of dietary restriction (post-hatch days 5–15), the last 15 days of dietary restriction (post-hatch days 16–30), and over the 40 days following the termination of the treatment (post-hatch days 30–70). Each random regression model included repeated measures of body mass and a fixed effect of diet (treatment versus control), an orthogonal polynomials of second order for age, and an interaction between the two. The second-order orthogonal polynomials include a centered and scaled linear effect of age and a centered and scaled quadratic effect of age. Thus, a significant interaction between treatment and the first-order or second-order polynomial for age would indicate differences in the linear or quadratic pattern of growth rate in control versus stressed females. Significance of fixed effects was tested with a conditional Wald *F*-statistic and the denominator degrees of freedom (df) were determined following Kenward and Roger (1997). To account for individual variation in body mass and growth rate (Schielzeth and Forstmeier 2009), we included a random effect for intercept, slope, and covariance between intercept and slope. We also allowed V_e to be estimated separately for control and stressed females (Cleasby and Nakagawa 2011). To obtain an overall estimate of the average growth rate in stressed and control birds during each period, we re-ran random regression models separately for stressed and control birds and extracted the estimate ($\pm$se) associated with the slope of the change in body mass across days.

We used univariate mixed models to test the effect of dietary restriction on the adult phenotype. Although body mass, hematocrit, and stressed-induced corticosterone level were not transformed, all behavioral and metabolic traits were $\log_{10}$-transformed to meet assumptions of normality of residuals. All traits were subsequently standardized to a mean of 0 and a variance of 1, such that the fixed and random-effect estimates obtained for different variables are directly comparable to each other. All models included fixed effects of early-developmental stress (treatment versus control), body mass, and measurement set (as a categorical variable). For behavioral variables, we also included a dummy categorical variable coding for the identity of the activity cage (one of eight). For metabolic variables, we included a dummy categorical variable coding for the identity of the metabolic chamber (one of four). To account for potential temporal changes in chamber-related differences, we included an interaction between chamber and measurement set. Partial regression plots were generated to visualize the effect of early-developmental stress on behavioral and physiological variables after conditioning on all other factors.

The V_P that the fixed effects did not take into account was partitioned into V_I (the variance attributed to individual identity as a random intercept) and the V_e (the residual variance). We first ran a "homogeneous" model in which both components of variance (i.e., V_I and V_e) were constrained to be equal in control and stressed birds, and this model

was used to calculate "pooled" repeatability estimates. We calculated R as the ratio of V_I to V_P (i.e., $V_I + V_e$) and the approximate standard errors for these estimates were obtained using the delta method (see appendix 1 in Lynch and Walsh 1998). We tested for the statistical significance of V_I using a log-likelihood ratio test (LRT) comparing the log-likelihoods of a full model that included V_I and a reduced model that excluded it. The LRT statistic is equal to twice the difference in log-likelihoods between the two nested models and is assumed to follow a χ^2-distribution with df equal to the difference in the number of parameters estimated. However, when testing a single component against a boundary of its parameter space (e.g., $V_I > 0$), the χ^2 statistic is distributed as an equally weighted mixture of χ^2-distributions with 1 and 0 df ($\chi^2_{0:1}$). In practice, this is equivalent to halving P-values obtained from a χ^2-distribution with 1 df (Snijders and Bosker 2012). We calculated the marginal and conditional R^2 for linear mixed models (R^2_{GLMM}) following Nakagawa and Schielzeth (2013). Although the marginal R^2 represents the amount of variance explained by fixed effects, the conditional R^2 can be interpreted as the variance explained by the entire model, with the difference between the two reflecting how much variability is due to random effects (Nakagawa and Schielzeth 2013).

In a second step, we ran a "heterogeneous" model in which both components of variance (i.e., V_I and V_e) were allowed to be different in control and stressed birds. We used the V_I and V_e estimates from this "heterogeneous" model to calculate repeatability in control and stressed birds separately. To test whether overall repeatability was different in control versus stressed birds, we used all repeatability estimates for behavioral and physiological traits and applied a two-tailed paired t-test. Because repeatability is influenced both by its numerator (V_I) and by its denominator ($V_I + V_e$), we tested whether V_I or V_e differed significantly between control and stressed birds. For each trait separately, we used a LRT with 1 df comparing the fully heterogeneous model to a model in which only V_I or V_e were constrained to be equal in control and stressed birds. Finally, we were also interested in testing the effect of developmental stress on V_P overall. To do this, we re-ran the "heterogeneous" model as above but after excluding V_I. Therefore, in this model the residual variance (V_e; after accounting for fixed effects) corresponds to V_P, which was partitioned separately in stressed and control groups. This allowed us to use a LRT with 1 df to test, for each trait separately, whether V_P was significantly different in control versus stressed birds.

Results

Effect of developmental stress on growth

Over the first 10 days of treatment (post-hatch days 5–15), growth rate was significantly reduced by dietary restriction (Table 1; "treatment × day" and "treatment × day^2" interactions). The average (±se) growth rate was lower in stressed birds ($0.432 \pm 0.052\,g\,day^{-1}$) than in control birds ($0.534 \pm 0.055\,g\,day^{-1}$), which resulted in a 6% difference in body mass (±se) at post-hatch day 15 (stressed birds: 9.72 ± 0.29 g, control birds: 10.31 ± 0.39 g).

For the rest of the duration of treatment (post-hatch days 16–30), the "treatment × day" interaction did not significantly affect growth rate (Table 1), with stressed birds ($0.138 \pm 0.021\,g\,day^{-1}$) and control birds ($0.132 \pm 0.024\,g\,day^{-1}$) growing at statistically indistinguishable rates. However, a significant and negative "treatment × day^2" interaction for this period (Table 1) indicated that body mass in control birds kept increasing, whereas body mass in stressed birds plateaued. At the end of the treatment (post-hatch day 30), there was a 4.5% difference in body mass (stressed birds: 11.94 ± 0.28 g; control birds: 12.48 ± 0.38 g).

Over the 40 days following the termination of the treatment (post-hatch days 30–70), the "treatment × day" and "treatment × day^2" interactions were not significant (Table 1). Yet, stressed birds had a slightly higher growth rate ($0.028 \pm 0.014\,g\,day^{-1}$) than did control birds ($0.016 \pm 0.007\,g\,day^{-1}$), which maintained over 40 days resulted in no difference in body mass at post-hatch day 70 (stressed birds: 12.63 ± 0.48 g, control birds: 12.48 ± 0.15 g). There was no significant difference in body mass at the time of behavioral and physiological measurements (stressed birds: 13.72 ± 0.20 g; control birds: 13.39 ± 0.23 g; $F_{1,17} = 0.37$, $P = 0.55$).

Effect of developmental stress on behavior and physiology

Descriptive statistics, including sample size, mean, and phenotypic variance are presented in Supplementary Table S1 for control and stressed individuals separately. Our main interest here was to look at the effect of early-developmental stress after accounting for other variables (i.e., body mass, measurement set, and dummy variables coding for individual activity cages and metabolic chambers), which are presented in Table 2. There was no difference between control and stressed

Table 1 Growth rate in female zebra finches subjected to early-developmental stress relative to control females

	Post-hatch days 5–15				Post-hatch days 16–30				Post-hatch days 30–70			
Fixed effects	**Estimate**	±	**se**	***P***	**Estimate**	±	**se**	***P***	**Estimate**	±	**se**	***P***
Intercept	7.98	±	0.46	<0.001	11.19	±	0.24	<0.001	12.84	±	0.40	<0.001
$Treatment_{[stressed]}$	−0.21	±	0.61	0.220	0.07	±	0.31	0.870	−0.16	±	0.54	0.455
Day	2.94	±	0.30	<0.001	0.95	±	0.17	<0.001	0.35	±	0.27	0.023
Day^2	−0.52	±	0.10	<0.001	0.33	±	0.09	0.003	−0.08	±	0.10	0.060
$Treatment_{[stressed]} \times day$	−0.78	±	0.39	0.070	0.02	±	0.23	0.847	0.19	±	0.36	0.557
$Treatment_{[stressed]} \times day^2$	0.45	±	0.13	0.001	−0.73	±	0.12	<0.001	−0.13	±	0.15	0.365
Random effects	**Estimate**	±	**se**		**Estimate**	±	**se**		**Estimate**	±	**se**	
V_I	5.2726	±	2.0727		2.2557	±	0.9981		1.7475	±	0.9554	
$COV_{I–S}$	−0.3022	±	0.1296		−0.0787	±	0.0372		−0.0319	±	0.0207	
V_S	0.0221	±	0.0091		0.0033	±	0.0015		0.0010	±	0.0005	
V_e (control)	0.2424	±	0.0440		0.2405	±	0.0358		0.1227	±	0.0444	
V_e (stressed)	0.1792	±	0.0283		0.1541	±	0.0202		0.1818	±	0.0557	

Notes: Shown are parameters from random regression models of body mass as the dependent variable and fixed effects of treatment (stressed versus control), linear slope for day, second-order quadratic function of day, and the interactions between these and treatment, in addition to random effects of intercept (V_I), slope (V_S), the covariance between intercept and slope ($COV_{I–S}$), and a heterogeneous residual–variances structure (V_e) according to treatment. The pattern of growth rate is described by the interactions between treatment and the linear and quadratic terms for day of measurement over three different phases of the development (post-hatch days 5–15, 16–30, and 30–70).

Table 2 Effects of early-development stress (treatment versus control), body mass, and measurement set (first, second, or third) on a suite of physiological and behavioral traits in female zebra finches, including activity and feeding in novel and familiar environments, hematocrit, stressed-induced corticosterone level, BMR, average MR in the morning (morning MR), and daytime RMR

	Early-development stress						Body mass						Measurement set		
Trait	**Estimate**	±	**se**	***F***	**df**	***P***	**Estimate**	±	**se**	***F***	**df**	***P***	***F***	**df**	***P***
Activity (novel environment)	−0.12	±	0.21	0.32	19.3	0.581	0.12	±	0.08	2.13	23.3	0.158	19.66	42.6	0.000
Activity (familiar environment)	−0.34	±	0.28	1.50	16.7	0.238	**0.31**	±	**0.12**	**6.66**	**25.4**	**0.016**	0.78	27.8	0.468
Feeding (novel environment)	**0.65**	±	**0.24**	**7.10**	**18.2**	**0.016**	**−0.29**	±	**0.09**	**9.57**	**31.4**	**0.004**	21.68	41.4	0.000
Feeding (familiar environment)	0.69	±	0.35	3.96	16.7	0.063	**−0.29**	±	**0.12**	**5.97**	**40.5**	**0.019**	5.39	26.6	0.011
Hematocrit	**0.83**	±	**0.31**	**6.88**	**16.4**	**0.018**	**0.20**	±	**0.11**	**3.51**	**44.8**	**0.067**	2.41	35.9	0.104
Corticosterone level	−0.29	±	0.35	0.72	16.0	0.408	0.17	±	0.13	1.69	35.4	0.203	0.37	35.8	0.695
BMR	**0.68**	±	**0.31**	**4.73**	**15.9**	**0.045**	0.13	±	0.12	1.16	35.0	0.288	6.98	28.3	0.003
Morning MR	**0.60**	±	**0.28**	**4.69**	**16.2**	**0.046**	0.08	±	0.10	0.57	36.4	0.454	36.53	28.4	0.000
RMR	−0.26	±	0.29	0.81	12.2	0.385	0.17	±	0.12	2.13	19.0	0.161	14.92	25.0	0.000

Notes: All traits were standardized to a mean of 0 and a total phenotypic variance of 1 (after transformation; see main text). The effect of developmental stress is expressed as treatment relative to control and bold numbers represent significance at $P < 0.05$. See Table 3 for random effects and repeatability.

females in activity rates either in novel or familiar environments (Table 2 and Fig. 4A and B). However, females of the stressed group showed a significantly higher rate of feeding in the novel environment (Fig. 4C and Table 2). Compared with control females, stressed females also showed higher feeding rates in the familiar environment (Fig. 4D), but the difference was marginally non-significant (Table 2). Compared with controls, stressed females had significantly higher hematocrit (Table 2 and Fig. 5A). Stress-induced corticosterone level, however, was not different in control versus stressed females (Table 2 and Fig. 5B). Finally, both BMR and morning MR were significantly higher in stressed than in control females (Table 2 and Fig. 5C and D).

Repeatability—pooled estimates

Using the homogeneous model to estimate and test the overall significance of the pooled repeatability estimates, we found that there was significant V_I in all traits except for activity in the novel environment

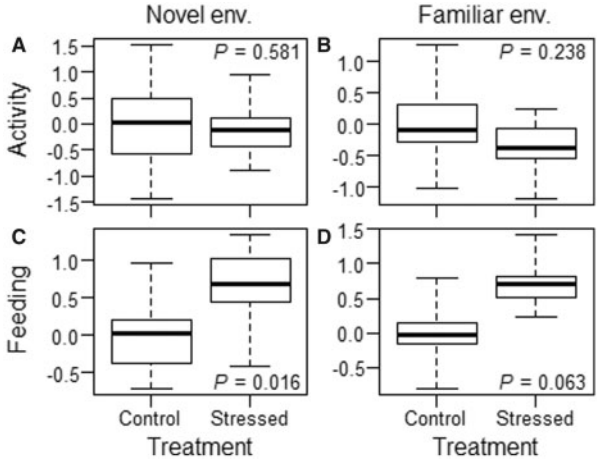

Fig. 4 Activity and feeding rates in a novel (panels **A** and **C**) and familiar (panels **B** and **D**) environment according to treatment category (control individuals versus those subjected to early-developmental stress, "stressed") in female zebra finches, shown as partial residuals which isolated the effect of treatment while accounting for all other covariates in the model. The median, 25th–75th percentiles, and range are indicated by the black line, box, and the whiskers, respectively.

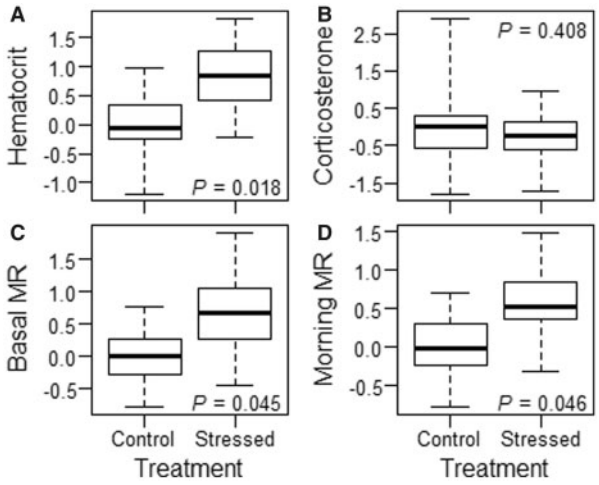

Fig. 5 (**A**) Hematocrit, (**B**) stress-induced corticosterone level, (**C**) BMR, and (**D**) morning MR according to treatment category (control individuals versus "stressed") shown as partial residuals which isolated the effect of treatment while accounting for all other covariates in the model. The median, 25th–75th percentiles, and range are indicated by the black line, box, and the whiskers, respectively.

and RMR ($P > 0.23$; Table 3). Repeatability estimates ranged from high for feeding in the familiar environment ($R = 0.77 \pm 0.09$), moderate for activity in the familiar environment, feeding in the novel environment, hematocrit, BMR, and morning MR (range: 0.37 ± 0.15–0.47 ± 0.15), low for stress-induced corticosterone level ($R = 0.28 \pm 0.16$), and very low for activity in the novel environment and RMR ($R < 0.18 \pm 0.22$).

Effect of developmental stress on repeatability

By modeling heterogeneous V_I and V_e in relation to treatment category, we could estimate repeatability of behavioral and physiological traits in control and stressed birds separately (Table 4). This revealed that, overall, R was significantly higher in stressed than in control birds (paired t-test: $t_8 = 5.193$, $P = 0.008$). Indeed, for any given trait, R was higher in stressed than in control birds (Fig. 6A; all points above the dashed line). Although the average R was 0.27 in control birds, it was 0.48 in stressed birds (Table 4).

Effect of developmental stress on among-individual and within-individual variance

Early-developmental stress did not significantly influence the level of *among*-individual variance in any trait (Table 4 and Fig. 6B). For the variable morning MR, however, there was a tendency for higher V_I in stressed than in control females ($P = 0.10$; Table 4). However, early-developmental stress did significantly influence the level of *within*-individual variance in activity in the novel environment ($P = 0.02$; Table 4 and Fig. 6C) and stress-induced corticosterone level ($P = 0.04$; Table 4 and Fig. 6C), and marginally affected BMR ($P = 0.08$; Table 4 and Fig. 6C).

Effect of developmental stress on phenotypic variance

Early-developmental stress had a variable effect on V_P. For some traits (activity in the familiar environment, feeding both in novel and familiar environments, and RMR), V_P was not significantly different in stressed versus control birds (Table 4 and Fig. 6D). However, V_P was significantly *higher* in stressed than in control birds for hematocrit, BMR, and morning MR (Table 4 and Fig. 6D). By contrast, V_P was significantly *lower* in stressed than in control birds for activity in the novel environment and stress-induced corticosterone level (Table 4 and Fig. 6D).

Discussion

In testing the effects of developmental stress both on absolute trait expression and on their variance within and among individuals, we demonstrate the capacity for stressors in early life to shape phenotypes at both individual and population levels. At the mean level, developmental stress did not affect activity or stress-induced corticosterone level, but did affect feeding

Table 3 Estimates (±se) of among-individual and within-individual variances (V_I and V_e, respectively) and repeatability (R) in a suite of physiological and behavioral traits in female zebra finches, including activity and feeding in novel and familiar environments, hematocrit, stressed-induced corticosterone level, BMR, average metabolic rate in the morning (morning MR), and daytime RMR

	Random effects								Repeatability			R^2_{GLMM}	
Trait	V_I	±	se	$\chi^2_{0:1}$	P	V_e	±	se	R	±	se	Marg.	Cond.
Activity (novel environment)	0.043	±	0.068	0.53	0.234	0.460	±	0.103	0.085	±	0.132	0.539	0.539
Activity (familiar environment)	**0.203**	±	**0.127**	**4.63**	**0.016**	0.322	±	0.091	**0.387**	±	**0.182**	0.538	0.717
Feeding (novel environment)	**0.170**	±	**0.092**	**8.54**	**0.002**	0.295	±	0.067	**0.366**	±	**0.145**	0.561	0.721
Feeding (familiar environment)	**0.493**	±	**0.198**	**23.84**	**0.000**	0.149	±	0.043	**0.768**	±	**0.092**	0.280	0.833
Hematocrit	**0.334**	±	**0.166**	**9.70**	**0.001**	0.381	±	0.092	**0.467**	±	**0.148**	0.307	0.631
Corticosterone level	**0.297**	±	**0.208**	**3.31**	**0.034**	0.762	±	0.184	**0.280**	±	**0.164**	0.057	0.322
BMR	**0.296**	±	**0.168**	**6.75**	**0.005**	0.402	±	0.110	**0.424**	±	**0.168**	0.423	0.668
Morning MR	**0.241**	±	**0.129**	**8.38**	**0.002**	0.287	±	0.078	**0.457**	±	**0.162**	0.531	0.745
RMR	0.137	±	0.174	0.56	0.226	0.606	±	0.175	0.184	±	0.217	0.371	0.487

Notes: All traits were standardized to a mean of 0 and a total phenotypic variance of 1 (after transformation; see main text). See Table 2 for fixed effects. The significance of V_I was tested using a LRT with a χ^2 statistic distributed as an equally weighted mixture of χ^2-distributions with 1 and 0 df ($\chi^2_{0:1}$); bold numbers represent significance at $P<0.05$. Also shown are the marginal and conditional R^2 estimated for general linear mixed models (R^2_{GLMM}).

rate, hematocrit, and BMR. Females that experienced dietary restriction early in their development not only showed higher BMR than did control females, but also had higher feeding rates and hematocrit. In addition, we demonstrated the insights afforded by taking multiple, repeated measures of pertinent variables in revealing the effects of early-developmental stress on the variance expressed at both among-individual and within-individual levels. In general, R was higher in stressed compared with control females, which was in part due to a higher V_I in most cases. We also found that early-developmental stress significantly reduced V_e in activity in the novel environment and stress-induced corticosterone level. Perhaps coincidentally, these two traits did not show differences in control versus stressed groups at the mean-response levels.

Effect of developmental stress on behavior and physiology

Previous research on developmental stress using the zebra finch as a study model have shown that provisioning parents have the capacity to adaptively "program" their offspring through developmental trajectories that set optimal behavioral and physiological strategies for later life (Buchanan et al. 2004; Spencer et al. 2005b; Criscuolo et al. 2008). Restriction of food early in the developmental phase reduces growth rate, the volume of the song control nuclei, and males' attractiveness (Buchanan et al. 2004; Spencer et al. 2005b), suggesting that poor developmental conditions reduce the expression of sexual traits in later life. Criscuolo et al. (2008) showed that individuals receiving low dietary protein levels during the nestling phase experienced compensatory growth, which resulted in a 19% elevation in BMR later in life. There are good reasons to expect early-developmental conditions to affect metabolism in later life, as pre-natal and post-natal restriction of protein in Wistar rats affects the development of liver, spleen, muscle, and pancreas, tissues essential for determining metabolic turnover (Desai et al. 1996). Other studies, however, have found no effect of early-developmental stress on adult BMR (Bech et al. 2004; Krause et al. 2009), including a recent study by Kriengwatana et al. (2013) who found age-specific effects of nutritional restriction on growth and immune function, but no effects of early-developmental stress on adult BMR. It must be noted, however, that the experimental manipulation employed by Kriengwatana et al. (2013) did not produce differences in growth rates in the early phase as was found in studies showing an effect of developmental stress on MR (this present study; Criscuolo et al. 2008; Schmidt et al. 2012).

The importance of ontogenetic studies of animal behavior has recently been highlighted (Stamps and Groothuis 2010), as consistent individual differences in several behavioral traits are likely to be affected by variation in developmental trajectories and subsequent physiological programming. Our data show that exposure to early-developmental stressors caused an increase in rate and/or duration of feeding behavior, plausibly linked to the increase in MR

Table 4 Repeatability (R), among-individual variance (V_I), within-individual variance (V_e), and phenotypic variance (V_P) in a suite of behavioral and physiological traits estimated for female zebra finches subjected to early-developmental stress (stressed) and their control

		Repeatability			**Among-individual variance (V_I)**					**Within-individual variance (V_e)**					**Phenotypic variance (V_P)**				
Trait	**Group**	R	±	se	V_I	±	se	χ^2_1	P	V_e	±	se	χ^2_1	P	V_P	±	se	χ^2_1	P
Activity (novel environment)	Control	0.060	±	0.165	0.046	±	0.134	0.00	0.952	**0.719**	±	**0.221**	**6.30**	**0.012**	**0.749**	±	**0.195**	**7.54**	**0.006**
	Stressed	0.166	±	0.200	0.037	±	0.053			**0.185**	±	**0.069**			**0.228**	±	**0.069**		
Activity (familiar environment)	Control	0.252	±	0.217	0.155	±	0.179	0.20	0.655	0.461	±	0.178	2.05	0.152	0.586	±	0.178	0.48	0.488
	Stressed	0.630	±	0.161	0.280	±	0.193			0.164	±	0.079			0.433	±	0.144		
Feeding (novel environment)	Control	0.231	±	0.181	0.090	±	0.092	0.86	0.353	0.300	±	0.098	0.00	0.947	0.402	±	0.109	0.62	0.432
	Stressed	0.467	±	0.171	0.254	±	0.174			0.291	±	0.099			0.546	±	0.154		
Feeding (familiar environment)	Control	0.736	±	0.108	0.530	±	0.294	0.01	0.914	0.190	±	0.078	0.67	0.414	0.724	±	0.220	0.61	0.434
	Stressed	0.830	±	0.080	0.487	±	0.275			0.100	±	0.050			0.513	±	0.171		
Hematocrit	Control	0.285	±	0.194	0.128	±	0.122	1.85	0.173	0.321	±	0.106	0.56	0.454	**0.439**	±	**0.120**	**4.16**	**0.041**
	Stressed	0.546	±	0.163	0.547	±	0.360			0.455	±	0.161			**0.976**	±	**0.281**		
Corticosterone level	Control	0.245	±	0.191	0.387	±	0.401	0.43	0.510	**1.194**	±	**0.403**	**4.19**	**0.041**	**1.557**	±	**0.424**	**7.03**	**0.008**
	Stressed	0.282	±	0.209	0.136	±	0.141			**0.347**	±	**0.132**			**0.473**	±	**0.144**		
BMR	Control	0.315	±	0.234	0.103	±	0.111	1.48	0.223	0.224	±	0.093	3.05	0.081	**0.313**	±	**0.107**	**5.89**	**0.015**
	Stressed	0.457	±	0.189	0.518	±	0.395			0.615	±	0.239			**1.149**	±	**0.361**		
Morning MR	Control	0.239	±	0.243	0.051	±	0.068	2.69	0.101	0.163	±	0.070	2.19	0.139	**0.205**	±	**0.071**	**7.38**	**0.007**
	Stressed	0.518	±	0.176	0.478	±	0.336			0.445	±	0.179			**0.942**	±	**0.293**		
RMR	Control	0.099	±	0.242	0.090	±	0.246	0.32	0.570	0.823	±	0.316	1.98	0.160	0.847	±	0.259	0.66	0.417
	Stressed	0.462	±	0.223	0.269	±	0.241			0.313	±	0.156			0.577	±	0.201		

Notes: Traits examined include activity and feeding in a novel and familiar environment, hematocrit, stressed-induced corticosterone level, BMR, average metabolic rate in the morning (morning MR), and daytime RMR. All traits were standardized to a mean of 0 and a total phenotypic variance of 1 (after transformation; see main text). For each trait separately, a LRT was used to test whether V_I, V_e, or V_P was significantly different between control and stressed groups, with a χ^2 statistic distributed with 1 df (χ^2_1). Bold numbers indicate significance at $P<0.05$.

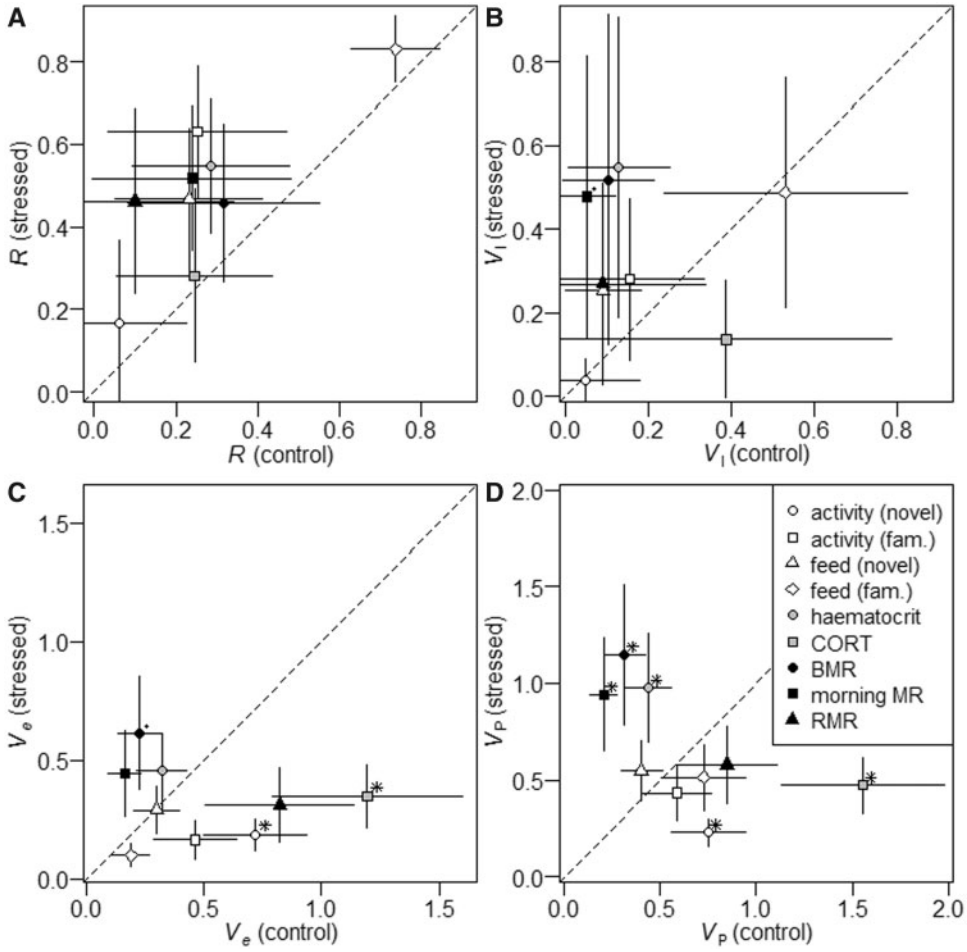

Fig. 6 (**A**) Repeatability ($R \pm$ se), (**B**) among-individual variance ($V_I \pm$ se), (**C**) within-individual variance ($V_e \pm$ se), and (**D**) phenotypic variance ($V_P \pm$ se) in a suite of behavioral and physiological traits estimated for female zebra finches subjected to early-developmental stress (stressed) versus their control. The dashed line represents the 1:1 line. For panels B–D, asterisks and small dots indicate estimates for which the difference between stressed versus control was significant ($P < 0.05$) or marginally non-significant ($P < 0.1$), respectively.

(Careau et al. 2008). Higher feeding frequency could be interpreted as an adaptive response that increases survival in an environment in which food is limited and unpredictable. Although our experimental design revealed the effect of early-developmental stress on the frequency of feeding, we found no difference in activity between treatment groups in the novel and familiar environments. Future studies should examine how developmental stress affects other "personality" traits (e.g., boldness and aggressiveness) and whether they are set from fledging or whether they can be modified over time by particular environmental or social interactions (Stamps and Groothuis 2010).

Our study did not find any effect of early-developmental stress on corticosterone level in response to a standardized capture and handling restraint. This may be because (1) there is no long-term effect of such early-developmental stress on the acute stress response, as has previously been reported (e.g., Spencer et al. 2005a), (2) the time point at which it was measured was not appropriate to detect this effect, or (3) the acute response is not the appropriate variable to measure. It is possible that individuals experiencing early-developmental stress may have chronically elevated basal corticosterone level (but see Honarmand et al. 2010), resulting in increased daily level. Such long-term elevations in basal corticosterone level could explain the increased feeding rate observed in stressed females (Astheimer et al. 1992), but not the increase in BMR (Buttemer et al. 1991). Our results also suggest that hematocrit was higher in individuals experiencing early-developmental stress, suggesting that these individuals may have had higher production of red blood cells, higher water loss, or lower intake of water.

However, the interpretation of hematocrit values can be problematic (Fair et al. 2007), as this measure is not sensitive to subtle changes in physical condition (Ots et al. 1998).

Repeatability in a suite of behavioral and physiological traits

Quantifying R is a key first step to any evolutionary analysis, as it indicates the reliability of multiple measurements on the same individual and it potentially sets an upper limit to h^2 (Falconer and Mackay 1996; but see Dohm 2002). Narrow-sense h^2 represents the proportion of V_P that is due to additive genetic variance responsible for the resemblance between parents and offspring. Thus, the efficiency at which natural selection can change trait-means over time can be much different in traits showing high, medium, or low R. Perhaps not surprisingly, an extensive literature has accumulated documenting repeatability of numerous physiological and behavioral traits in a great diversity of organisms (Versteegh et al. 2008; Bell et al. 2009; Careau and Garland 2012; Wolak et al. 2012; White et al. 2013).

Our pooled R estimates for the four behavioral traits ranged from relatively low ($R = 0.09 \pm 0.13$) to high ($R = 0.77 \pm 0.09$), but on average, R in our behavioral measures was close ($R = 0.40$) to the average R of behavioral traits measured across taxa ($R = 0.37$; Bell et al. 2009). By contrast, our pooled R estimates for the three metabolic traits ranged from relatively low ($R = 0.18 \pm 0.22$) to moderate ($R = 0.46 \pm 0.16$), but on average, the R we obtained for metabolic traits ($R = 0.36$) was slightly lower than the average R reported for MR across taxa ($R = 0.46$; White et al. 2013).

In our sample, stress-induced corticosterone level was repeatable at $R = 0.28 \pm 0.16$, which is lower than previously reported ($R = 0.41$) in female zebra finches between the nestling and adult stages (Wada et al. 2008). Few estimates of R are available for circulating hormone levels in general, but data on this are accumulating rapidly (Williams 2008; Ouyang et al. 2011). There also are few estimates of R for hematocrit measurements in birds. In a large sample of wild adult blue tits (*Cyanistes caeruleus*), for example, R of hematocrit was $R = 0.28 \pm 0.05$ (Kluen et al. 2014). In great tits, R of hematocrit ranged from $R = 0.32 \pm 0.15$ in spring to $R = 0.10 \pm 0.08$ across years (Norte et al. 2008). In female pied flycatchers (*Ficedula hypoleuca*), R of hematocrit was 0.23 ± 0.09 for females and 0.17 ± 0.11 for males (Potti 2007). Thus, our estimate of R for hematocrit (0.47 ± 0.15), obtained for captive birds held under consistent environmental conditions, was considerably higher than previously reported in studies of wild birds (see also Hatch and Smith 2010).

Surprisingly few studies on early-developmental stress have included repeated measures in their experimental design and an evaluation of R. Yet, any conclusion that early-developmental stress causes physiological and behavioral programming requires that the effects on such traits persist over a significant period of time trough adulthood. Moreover, the interpretation that such programming is "adaptive" requires that these changes ultimately lead to improved lifetime reproductive success. As this has not yet been convincingly demonstrated, we suggest that future studies include gathering repeated measures, not only to increase precision, robustness, and evolutionary relevance, but also to quantify the effect of early-developmental stress on R *per se*.

Effect of developmental stress on repeatability and among-individual variance

We found that overall, R of our behavioral and physiological measures was 77% higher in stressed (mean $R = 0.48$) than control groups (mean $R = 0.27$). One possibility is that the higher R we observed is due to a higher h^2 in stressed than control females. Indeed, developmental stress could cause cryptic genetic variation that is already present, but not expressed, to suddenly be expressed as phenotypic variation (Debat and David 2001; Flatt 2005). Exposure of such new heritable phenotypic variation could facilitate adaptive evolution in "stressful" environments. Although we did not estimate h^2, this scenario is supported by the observation that V_I was higher in stressed than control females for six out of nine traits examined (Fig. 6B). The average V_I was 0.33 and 0.18 in stressed and control females, respectively (Table 4).

However, V_I also includes non-additive sources of variance (dominance and epistasis) and permanent environmental effects (Wilson et al. 2010). Thus, any increases in these causal components of variance could also be responsible for the higher V_I in stressed versus control females. To test the hypothesis that developmental stress releases cryptic genetic variation, one could use special breeding designs in quantitative genetics to verify whether V_A increases in a population experiencing developmental stress, or use molecular-genetics tools to identify polymorphic loci that have no effect on phenotypes until they are perturbed by developmental stress (Paaby and Rockman 2014). Another possibility would be to

apply artificial selection in stressed and control groups, and quantify whether the rate of evolutionary response is faster in stressed versus control groups, as might be predicted.

Effect of developmental stress on within-individual variance

Another possible explanation for the higher R in stressed than in control females is a reduction in V_e. Indeed, this scenario seems to contribute to the overall pattern in R, because, on average, V_e was lower in stressed than in control females (0.32 and 0.49, respectively; see also Fig. 6C). In fact, V_P was not different in stressed versus control females (0.65 and 0.65, respectively; see also Fig. 6D).

Although we did not find any general effect of early-developmental stress on V_e, an examination of this component in stressed and control females for each trait separately revealed that early-developmental stress significantly reduced V_e both in activity in the novel environment and in stress-induced corticosterone level. This reveals the importance of simultaneously considering individual and mean-response levels; had we only focused on mean-response levels we would have concluded that early-developmental stress does not have an effect on these traits. The V_e component includes sensitivity to micro-environmental (localized) effects that are specific to each individual within a group and affect the consistency of individual responses. Hence, our results show that early environmental stress reduced the sensitivity of individuals to micro-environmental variation later in their life (Flatt 2005). As such, this suggests that early-developmental stress acted to canalize stress-induced corticosterone level and activity in the novel environment at the micro-environmental level.

Conclusions

Our results not only confirm previous findings on the effect of early-developmental stress on BMR (Criscuolo et al. 2008; Schmidt et al. 2012), but also extend the effect of stressors in early life to feeding rate and hematocrit. These results suggest that the developmental plasticities in these traits may be ontogenetically linked, or functionally integrated (Cheverud 1982), such that individuals have the capacity to co-adjust multiple aspects of their phenotype to environmental conditions. Our study reveals the potential for early-developmental conditions to affect behavioral and physiological strategies in a manner that adaptively programs individuals for the particular environmental conditions they will encounter.

Our results demonstrate the potential for environmental stress to influence developmental trajectories, releasing individuals from a restricted range of developmental outcomes. To study/demonstrate canalization, however, the amount of genetic variation must be controlled between lines/populations; multiple, independent genotypes (not individuals) must be sampled; and genetic background must be controlled for comparisons between treatments (Dworkin 2005). Still, our study highlights the role that environmental stress may play in generating variance at the among-individual and within-individual levels, hence potentially influencing the rate of evolution by changing the variation on which selection can act (Wagner and Altenberg 1996). The possibility that dietary restriction early in development releases cryptic genetic variation warrants future research. Given the number of studies that have examined changes in gene expression under environmental stress, there is potential for examining the robustness of these transcriptional changes across taxa.

Another interesting avenue for future research will be to look at the correlations among behavioral and physiological variables and test whether the strength of these relationships is different in groups experiencing different degrees of developmental stress. If the decanalization scenario turns out to be correct, then there is no reason to expect that the released genetic variation will cause identical associations between physiological and behavioral traits. Therefore, it is possible that correlations between behavior and MR (Careau et al. 2008; Careau and Garland 2012) are obscured or accentuated by early-developmental stress.

Acknowledgments

The authors thank J. Evans for running the treatments during the breeding of the birds, R. Collins for taking care of the birds, and S. Lisovski and M. Berg for help with gathering blood samples.

Funding

This work was supported by funding from the Centre for Integrative Ecology [to W.A.B.], an Alfred Deakin postdoctoral research fellowship [to V.C.], and an Australian Research Council grant [DP130100417 to K.L.B.].

Supplementary data

Supplementary Data available at *ICB* online.

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