Adrenocortical responses in zebra finches (*Taeniopygia guttata*): Individual variation, repeatability, and relationship to phenotypic quality

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Abstract

Although individual variation is a key requirement for natural selection, little is known about the magnitude and patterns of individual variation in endocrine systems or the functional significance of that variation. Here we describe (1) the extent and repeatability of inter-individual variation in adrenocortical responses and (2) its relationship to sex-specific phenotypic quality, such as song duration and frequency and timing of egg laying. We measured adrenocortical responses to a standardized stressor in zebra finches (*Taeniopygia guttata*) at two life history stages: ~day 16 (nestlings) and 3 months of age (sexually mature adults). Subsequently, we assessed phenotypic (reproductive) quality of all individuals as adults. Marked inter-individual variation in the adrenocortical response was seen in both sexes and ages, e.g., stress-induced corticosterone ranged from 2.2 to 62.5 ng/mL in nestlings and 5.0–64.0 ng/mL in adults. We found sex differences in (a) inter-individual variation in the adrenocortical response, (b) repeatability, and (c) relationships between corticosterone levels and phenotypic quality. In males, variation in nestling corticosterone was weakly but positively correlated with brood size and negatively correlated with nestling mass (though this relationship was dependent on one individual). There was no significant correlation of adrenocortical responses between two stages in males and adult phenotypic quality was significantly correlated only with adult corticosterone levels. In contrast, in females there was no relationship between nestling corticosterone and brood size or mass but adrenocortical response was repeatable between two stages ($r^2 = 0.413$). Phenotypic quality of adult females was correlated with nestling baseline and adrenocortical response.
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Introduction

Although it is a widely held view that there is an extensive physiological diversity among individuals of the same species (e.g., Adkins-Regan, 2005), it is still very rare for studies to present, let alone formally analyze, inter-individual variation in endocrine or physiological traits (Bennett, 1987; Williams, in press). Thus, we currently have a very poor understanding of the magnitude and general patterns of inter-individual variation (but see Spicer and Gaston, 1999) and the functional consequences of this variation. For instance, little is known about the extent to which inter-individual variation in hormonal traits explains variation in other hormonally mediated phenotypic traits or how it contributes to variation in fitness (Whitehead and Crawford, 2006; Zera et al., 2007). Clearly such an understanding is very important, especially for the integration of endocrinology and physiology with disciplines such as evolutionary biology (e.g., Ketterson and Nolan, 1999; Zera and Harshman, 2001): heritable, repeatable, inter-individual variation is a fundamental requirement for evolution by natural selection. For discontinuous traits, such as with many behaviors (Adkins-Regan, 2005) or polymorphisms (e.g., among reproductive or mating morphs; Oliveira et al., 2001; Sinervo and Calsbeek, 2003), clear dose–response relationships between hormone titer and phenotype

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have been documented, though these are typically non-linear and take the form of threshold or step functions (Adkins-Regan, 2005). It is far less clear how individual variation in hormone titers relates to variation in continuous traits (or variation within specific behaviors or morphs), although it has been suggested that for behavioral traits these correlations can often be near zero or even negative (Adkins-Regan, 2005).

Here we describe inter-individual variation and repeatability in hypothalamic–pituitary–adrenal (HPA) axis reactivity to a standardized stressor in nestling and adult zebra finches (Taeniopygia guttata) and relate this variation to sex-specific measures of phenotypic quality. Mammalian and avian studies have shown that early hormonal and behavioral experiences have permanent effects on glucocorticoid-driven stress physiology: pre- and post-natal stress results in enhanced stress reactivity later in life (reviewed in Caldji et al., 2001). In addition, variation in adrenocortical response in adults is widely assumed to have fitness consequences. For instance, glucocorticoids have been negatively correlated with foraging success (Angelier et al., 2007), numbers of offspring successfully raised (Bonier et al., 2007; Good et al., 2005), and survival (Blas et al., 2007; Romero and Wikelski, 2001) (but also see Comendant et al., 2003). Several studies have described inter-individual variation in the adrenocortical response of adult animals (birds, Littin and Cockrem, 2001; Cockrem and Silverin, 2002; mammals, Guimont and Wynne-Edwards, 2006) and individual variation in adrenocortical response can be repeatable (Cockrem and Silverin, 2002; Schjolden et al., 2005). Furthermore, in hamsters post-stress corticosterone (CORT) levels are positively correlated with pre-stress baseline CORT suggesting that individual variation in "baseline", non-manipulated hormone levels can be informative in terms of individual variation in subsequent activation of the HPA axis (Guimont and Wynne-Edwards, 2006). However, no studies have correlated inter-individual variation across different life history stages (e.g., are adrenocortical responses fixed during development or do they remain plastic?), and there are few data available to investigate the nature of correlations between variation in adrenocortical response and phenotypic quality (but see Romero and Wikelski, 2001).

In this study, we used a repeated measure design to characterize the acute adrenocortical response to stress in 16-day-old nestlings and then again, in the same birds, as adults. We measured baseline CORT and max CORT and calculated integrated CORT (CORT secreted during the 30 min) and the rate of increase in CORT during the first 15 min of the adrenocortical response; we measured total hormone levels (both bound to binding proteins and unbound) and estimated free (unbound) levels of steroid hormone. We had three specific objectives: (1) to describe the extent of inter-individual variation at each life-history stage; (2) to determine if the adrenocortical response was repeatable or plastic, i.e., the extent to which variation in the adrenocortical response of nestlings predicted their response as adults; and (3) to assess whether inter-individual variation in adrenocortical response, either for nestlings or adults correlated with other measures of sex-specific phenotypic quality: mating and courtship performance in males and fecundity or egg-laying ability in females.

Materials and methods

The experimental study was carried out between February and August 2005 using a captive zebra finch colony (T. guttata) at Simon Fraser University, Burnaby, British Columbia, Canada. Animal care and husbandry protocols were approved by Canadian Committee on Animal Care (CCAC) and Institutional Animal Care and Use Committees (IACUC). General diet and housing protocols are described in Williams (1996) but briefly randomly selected pairs were allowed to breed in individual breeding cages ($61 \times 46 \times 41$ cm) equipped with an external nest box ($15 \times 14.5 \times 20$ cm). Birds were provided with a standard Panicum seed diet (Just for Birds, Surrey, BC, Canada) ad lib and an egg food supplement (6 grams; 20.3% protein, 6.6 % lipid) daily between pairing and clutch completion and during chick rearing. Nest boxes were checked daily between 0900 and 1100 h to determine laying interval (i.e., days between pairing and initiation of egg laying), egg mass, clutch size, and brood size at hatching and fledging. Chicks were separated from their parents at c. 30 days of age (once they were feeding independently) and were held in large, mixed-sex non-breeder cages until development of sex-specific plumage at which time they were housed in large, single sex cages.

Stress series

Individuals in this experiment were subjected to a standardized restraint stress protocol (Wingfield, 1994; see below) during two life-history stages; first at ∼16 days (∼2 days before fledging) and again at 3 months of age (as sexually mature adults; Zann, 1996). This does not mimic natural stressors but rather examines the status of individuals' HPA axis using a common stressor. Serial blood samples were taken within 3 min (baseline), at 15 min, and 30 min of either opening the nest box (nestlings) or reaching into the cage (adults). Individuals were held in an opaque cloth bag between sampling. Body mass was measured immediately following collection of the baseline sample, while all other measurements (tarsus, wing length, head, back, and tail length) were taken after collection of the 30-min sample. Blood samples were kept in a cooler until transport to the lab, where samples were spun for 10 min at 5000 rpm. Plasma

Table 1
Extent of individual variation in HPA axis reactivity

		Min	Max	Mean	95% CI		CV%	n
					Lower	Upper		
(a)								
Nestling	Total 0	0.60	9.02	1.77	1.26	2.27	86.42	38
	Total 15	5.68	46.40	21.88	19.04	24.72	39.49	38
	Total 30	2.18	62.50	20.73	16.16	25.29	67.00	38
	Free 0	0.01	0.13	0.04	0.03	0.05	64.86	37
	Free 15	0.09	2.55	0.89	0.71	1.07	61.80	38
	Free 30	0.04	3.25	0.81	0.58	1.05	87.04	37
Adult	Total 0	0.75	6.84	2.09	1.68	2.51	60.29	38
	Total 15	4.11	49.70	12.82	10.21	15.44	62.09	38
	Total 30	4.97	64.00	15.52	12.02	19.03	68.62	38
	Free 0	0.01	0.09	0.03	0.02	0.03	59.00	38
	Free 15	0.04	1.37	0.28	0.20	0.37	95.36	38
	Free 30	0.07	4.68	0.53	0.23	0.84	177.74	38

		Nestling	Adult	Average
(b)				
Males	Total CORT	68.13	78.10	73.11
	Free CORT	77.76	128.17	102.96
Females	Total CORT	47.11	41.70	44.40
	Free CORT	56.36	99.00	77.68

(a) Mean corticosterone levels (ng/ml; both total and free corticosterone) with distribution around the mean. 95% confidence intervals (CI), coefficient of variation (CV), and sample size (n) are listed. (b) Averaged coefficient variation (0, 15, and 30) for both sexes.

was then separated and stored at −80 °C until they were shipped on dry ice to the University of Texas at Austin, where they were analyzed for CORT and corticosteroid binding globulin (CBG).

Assessment of phenotypic quality of adults

Upon completion of the adult stress series at 3 months of age, one female and one male per brood were selected (matched for body mass and age) for a breeding attempt with an experienced male, or a courtship trial with an experienced female, respectively. For each individual, we conducted two breeding or courtship trials so we could determine repeatability of phenotypic traits that would be reliable indicators of individual quality.

Each selected female was paired and allowed to breed with a randomly chosen experienced male under standard conditions as described above. We recorded laying interval (i.e., days between pairing and initiation of egg laying), egg mass, and clutch size. In addition, blood samples were collected from each female on the day the first egg was laid to measure plasma yolk precursor levels (vitellogenin (VTG) levels measured as plasma zinc levels, and very low density lipoprotein (VLDL) levels measured as plasma triglyceride levels; following Mitchell and Carlisle, 1991; Williams and Christians, 1997). Clutches were considered complete following 2 days of no additional eggs, and we then rested females for approximately five weeks before repeating the breeding trial.

Male courtship trials were conducted as follows: an experienced wild-type female was randomly chosen and placed in a cage (61×46×41 cm) for 5 min to acclimate alone. Different females were randomly chosen for each male and trial. The cage contained a perch, a grit, a cuttlefish bone, but no water or food inside, and a microphone and timer on the outside. The cage was visually but not acoustically isolated from other cages. Each male in this study was placed in the

cage with the experienced female, and the behaviors of both the male and the female were recorded for 15 min. All of the courtship trials were performed between 0900 and 1200 h. Six typical male courtship displays (described in Zann, 1996) were recorded during the experiment: invitation (Y or N), bill wiping (number of wipes), head or tail twisting (scored per left to right cycle), following (number of times the male followed the female), singing (duration and frequency), and mounting/copulation (number of mount, number of successful copulation, and time in seconds to initial attempt).

Plasma corticosterone and CBG assays

Corticosterone Enzyme Immunoassay kits from Assay Designs (catalog # 901-097) were used to measure plasma CORT levels (described in Wada et al., 2007). Samples were run in duplicate with 1:40 dilution and 1.5% steroid displacement buffer (Wada et al., in review). All 6 samples from one individual were run on the same plate; however each plate had randomly selected individuals and all samples within each plate were also randomized. Detection limit of the assay was 0.02 ng/well (0.8 ng/ml). Intra- and inter-assay variations were 6.1% and 11.7%, respectively.

Plasma CBG levels were measured using radio-ligand binding assay (described in Breuner et al., 2003). Individual CBG levels were measured in point sample assays where non-specific bindings (known amount of 3 H CORT, cold CORT, and plasma) were subtracted from total bindings (known amount of 3 H CORT, buffer, and plasma) to obtain specific bindings. Optimal plasma dilution of 1:1620 and incubation of 2 h at 4 °C were used (Wada et al., in review). In the point sample assays, each plasma sample was stripped then diluted with assay buffer and separated into two total binding and two non-specific binding tubes. Total binding was measured using 50 μL 3 H CORT,
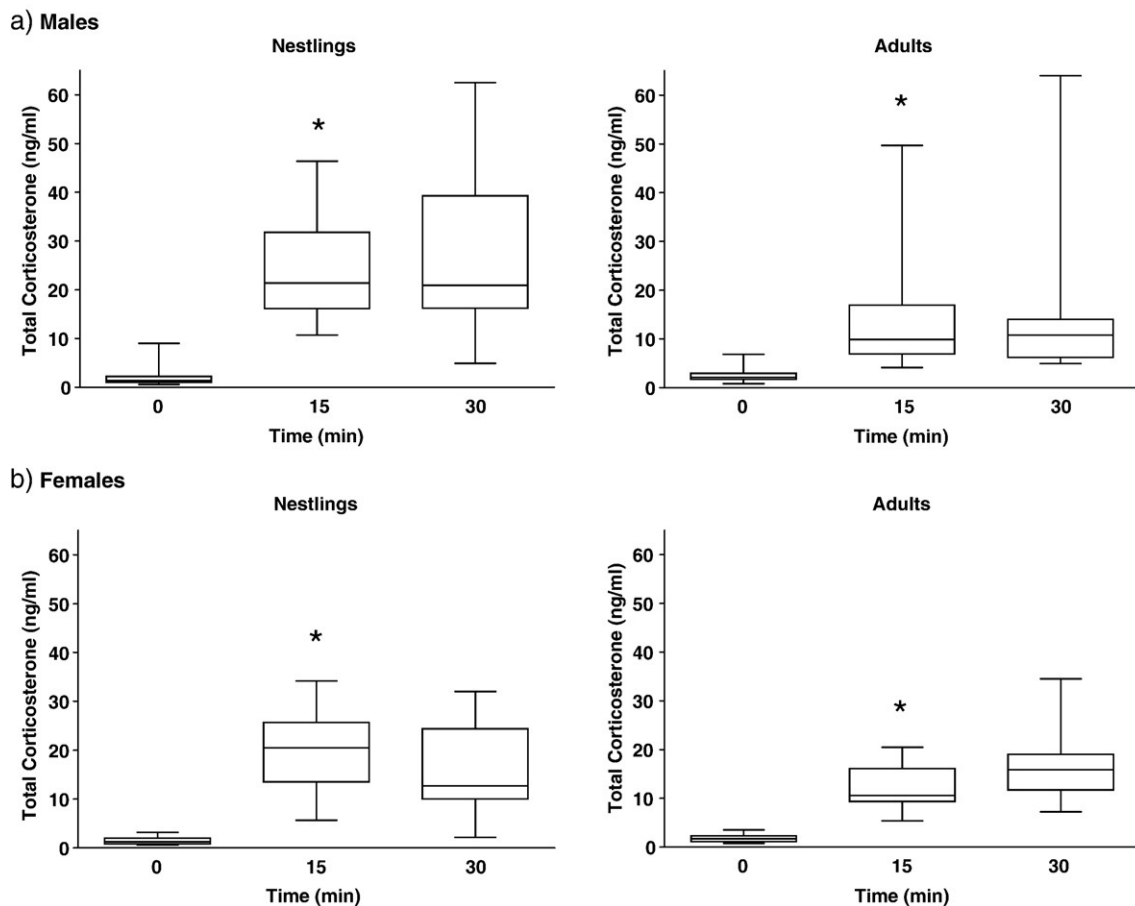

Fig. 1. Adrenocortical responses of male (a) and female (b) zebra finches (only total corticosterone levels are shown here). Boxes represent 25th to 75th percentiles with a line at the median. Whiskers represent the minimum and maximum values. Asterisks indicate significant differences between nestling and adult levels.

50 μL buffer, and 50 μL plasma, while non-specific binding was measured using 50 μL 3 H CORT, 50 μL 1 μM cold CORT, and 50 μL plasma. After the 2-h incubation, samples were filtered with chilled rinse buffer to trap 3 H CORT-bound CBGs, which were later counted for their radioactivity. All the samples were randomly distributed among three point sample assays that were run in two consecutive days. An average of 17.81 nM 3 H CORT was used in these assays, which should occupy 77.6% (nestlings) and 90.3% (adults) of total binding sites. Thus, we adjusted the CBG capacity to 100% for the free hormone analysis. Intra- and inter-assay variations were 9.3% and 18.5%, respectively.

To estimate the free hormone levels, we used the equation by Barsano and Baumann (1989):

$$H_{\text{free}} = 0.5$$
$$\times \left[H_{\text{total}} - B_{\text{max}} - 1/K_a \pm \sqrt{(B_{\text{max}} - H_{\text{total}} + 1/K_a)^2 + 4(H_{\text{total}}/K_a)} \right]$$

where K_a is $1/K_d$ (nM), K_d is affinity of CORT for CBG, B_{max} is total CBG capacity, and H_{total} is total plasma hormone concentration. K_d was determined in equilibrium binding analysis using pooled plasma (Wada et al., in review).

Table 2
Summary of multiple regression analysis for males (a) and females (b) with environmental and body condition factors against corticosterone levels

		Model summary		Factors	Standardized beta	Significance
		Adjusted r^2	Significance			
(a) Males						
Nestling	Total baseline	0.483	0.006	Brood size	0.368	0.058
				Nestling mass	−0.506	0.02
				Nestling tarsus		ns
	Total integrated	0.046	0.323	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free baseline	0.474	0.01	Brood size	0.411	0.047
				Nestling mass	−0.527	0.019
				Nestling tarsus		ns
	Free integrated	−0.019	0.466	Brood size		
				Nestling mass		
				Nestling tarsus		
Adult	Total baseline	0.153	0.103	Adult mass		
				Adult tarsus		
	Total integrated	0.199	0.066	Adult mass		
				Adult tarsus		
	Free baseline	0.1	0.167	Adult mass		
				Adult tarsus		
	Free integrated	0.277	0.029	Adult mass		ns
				Adult tarsus	−0.451	0.08
(b) Females						
Nestling	Total baseline	−0.182	0.943	Brood size		
				Nestling mass		
				Nestling tarsus		
	Total integrated	0.007	0.406	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free baseline	−0.183	0.945	Brood size		
				Nestling mass		
				Nestling tarsus		
	Free integrated	−0.188	0.956	Brood size		
				Nestling mass		
				Nestling tarsus		
Adult	Total baseline	−0.082	0.659	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Total integrated	−0.036	0.518	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Free baseline	−0.159	0.91	Adult mass loss		
				Adult mass		
				Adult tarsus		
	Free integrated	−0.188	0.985	Adult mass loss		
				Adult mass		
				Adult tarsus		

Adult mass and tarsus are the average values of trials 1 and 2, which were approximately 1.5 months apart. Standardized beta refers to regression coefficients for standardized data that represents how much each variable contributes to the model. No factor analysis is presented when the model lacked significance. ns=non-significant.

Data analysis

Statistical analyses were performed using SPSS 15.0 and JMP 5.0.1. Multiple regression analyses examined relationships between CORT and possible factors influencing the individual variation in HPA axis reactivity. Repeatability of the adrenocortical response (log-transformed) and reproductive traits were determined using the equation by Lessells and Boag (1987). Effects of age and handling on total and free CORT as well as CBG were determined by repeated measures ANOVA. For the nestling samples, effects of actual age on total CORT were determined by one-way ANOVA. To compare CORT levels of both sexes with varying reproductive quality, we first examined whether any of the CORT variables measured were correlated with each other. This ensured a selection of specific CORT measures that more accurately represented individuals' stress physiology. For both total and free levels, maximum CORT, integrated CORT (calculated as the area under the adrenocortical response curve), and rate of increase within 15 min were all significantly correlated with each other ($p < 0.05$), with the exception of maximum free CORT and the rate of increase in free CORT of adult females. Therefore, only baseline and integrated total and free CORT were examined in relation to reproductive quality. We then averaged the two breeding or courtship trials, took the upper and lower thirds of the distribution of each of the reproductive measures, and designated them as "high" and "low quality" individuals. We compared CORT levels of "high" and "low" quality individuals using MANOVAs. Identical plasma pools yielded different CBG levels in three separate assays. The CBG levels of individual samples were adjusted according to the differences between assays. When CORT exceeds CBG levels, it results in supra-physiological levels of free CORT. This occurred only in one out of 114 samples, and this sample was excluded from further analysis. When CORT was below detectabilities of the assay, the detection limit of the plate was assigned (detection limit for CORT assay was calculated as two

standard deviations away from the total binding wells). Levene's and Bartlett's tests were used to test for homogeneity of variances prior to all analyses. Data are presented as mean±SE.

Results

Individual and age-related variation in corticosterone and CBG levels

There was large inter-individual variation in plasma CORT for both 16-day nestlings and adult at 0, 15, and 30 min (Table 1a and Fig. 1). Total baseline and stress-induced levels (30 min) of CORT varied between 0.60 and 9.02 ng/mL and between 2.18 and 62.5 ng/mL, respectively. Free CORT varied between 0.01 and 0.13 ng/mL and between 0.07 and 4.68 ng/mL for baseline and stress-induced levels, respectively. In other words, total CORT showed as much as 15- and 29-fold differences between individuals in baseline (nestlings) and stress-induced levels (nestlings), respectively. Free CORT showed a maximum 12-fold difference in baseline (nestlings) and 70-fold difference in stress-induced levels (adults). Interestingly, average coefficients of variation for total and free CORT were always higher in males than females at both ages (Table 1b).
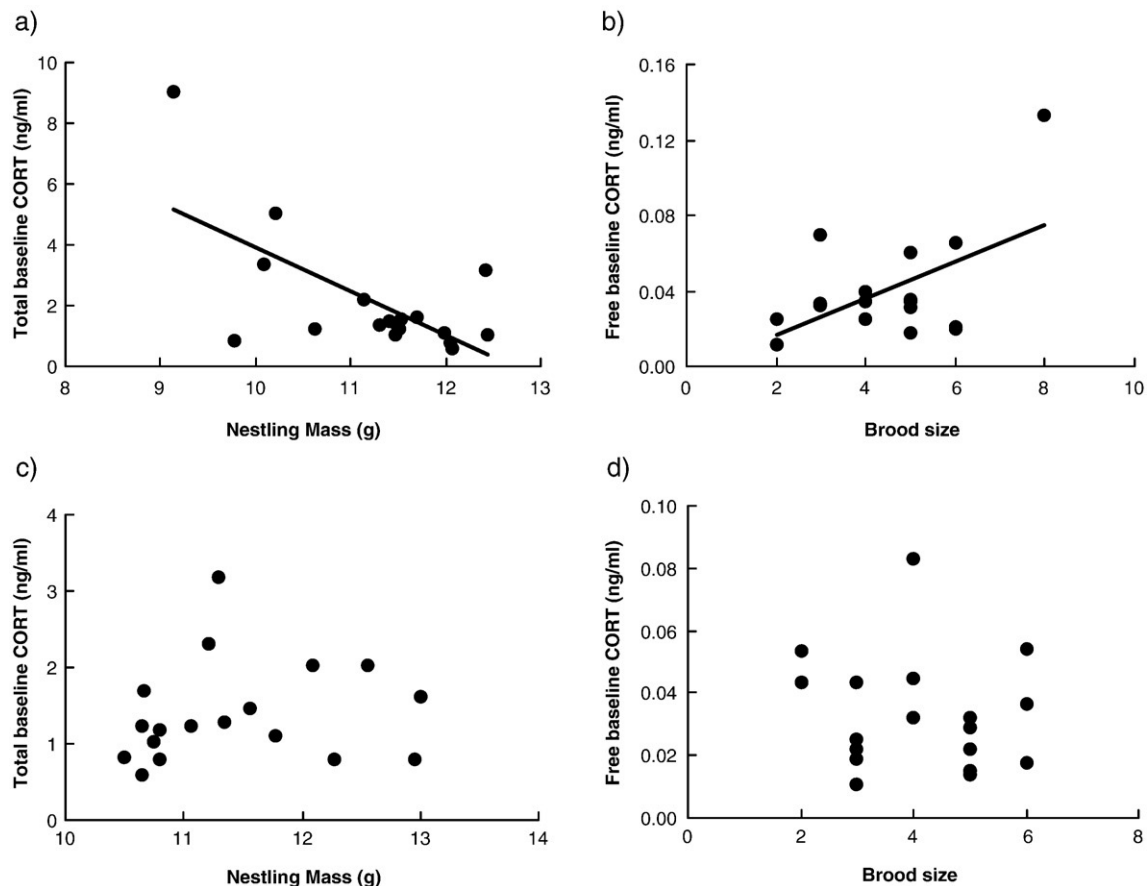

Fig. 2. Factors contributing to individual variation in corticosterone levels. In males, both nestling mass and brood size significantly correlated with nestling total (adjusted $r^2=0.483$, $p=0.006$) and free (adjusted $r^2=0.474$, $p=0.01$) baseline corticosterone levels (a and b); however, removal of the one outlier leaves a non-significant result in both mass and brood size data sets ($p>0.20$). No significant relationship was seen in females (c and d).
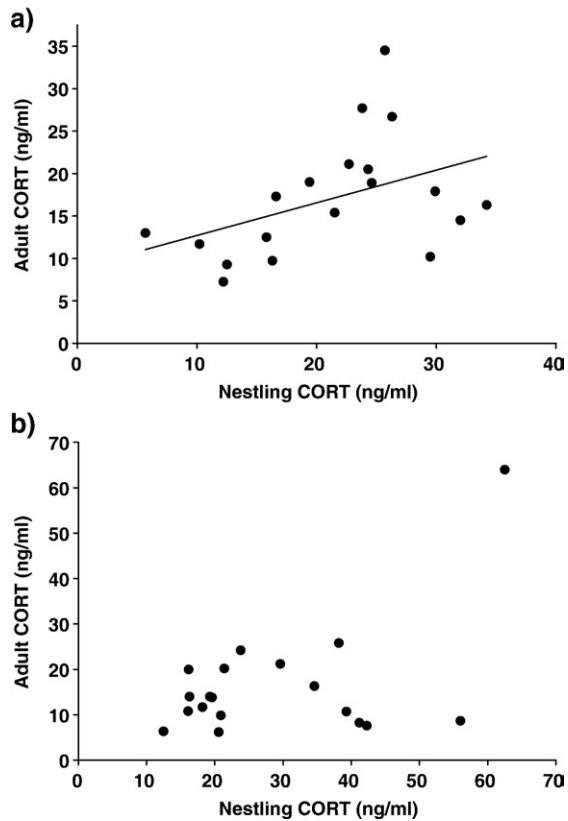
Fig. 3. Repeatability of inter-individual variation in the adrenocortical response. Female total max corticosterone (a; adjusted $r^2 = 0.413$, $p = 0.03$) was repeatable across age, while others such as male total max CORT (b; $p > 0.20$) was not.

Multiple regression analyses showed that in males both total ($p = 0.02$) and free ($p = 0.019$) baseline CORT were negatively correlated with nestling mass (Table 2 and Fig. 2). In contrast, both total ($p = 0.058$) and free ($p = 0.047$) baseline CORT were positively correlated with brood size. However, these relationships are largely driven by one extreme data point. Excluding this value these correlations were all non-significant ($p > 0.20$). Once males reach adulthood, adult tarsus length was marginally correlated with free integrated CORT ($p = 0.08$), but other measures of body condition and body mass were independent of hormone levels. In females, none of the hormone levels correlated with nestling "environmental" or "body condition" factors at either age (brood size, mass, mass loss during breeding, and tarsus; $p > 0.10$ in all cases).

There was some evidence for repeatability of inter-individual variation in adrenocortical response between nestling and adult stages, but only females and in certain measures of CORT. Total max CORT was repeatable (adjusted $r^2 = 0.413$, $p = 0.030$; Fig. 3a), and total integrated CORT was marginally repeatable in females (adjusted $r^2 = 0.282$, $p = 0.105$), while other traits were not repeatable between 16 days and 3 months of age (Fig. 3b). None of the free CORT measures were repeatable.

Zebra finches had significantly higher circulating levels of total CORT (bound and free CORT, Fig. 1, $F = 9.23$, $p = 0.003$), CBG ($F = 12.32$, $p = 0.001$), and free CORT ($F = 12,49$, $p = 0.001$) as ∼ 16-day-old nestlings than as 3-month-old adults. However, there was no significant effect of sex ($p > 0.05$), and

there was no interaction between age and sex ($p > 0.05$) for any of the CORT or CBG measurements. Pairwise comparisons showed that nestlings had significantly higher levels of total and free CORT at 15 min and at 0 and 15 min for CBG ($p < 0.05$). In addition, total baseline ($F = 0.160$, $p > 0.10$), max CORT ($F = 2.22$, $p > 0.10$), or integrated CORT ($F = 1.97$, $p > 0.10$) did not differ significantly among 15-, 16-, and 17-day-old nestlings.

Variation in corticosterone levels in relation to phenotypic quality

Prior to analysis of variation in hormone levels, we selected fecundity or mating traits that we considered good indicators of an individuals' phenotypic quality: selected traits were either (1) found to be repeatable in our study between trials or (2) have been shown to be repeatable in previous studies. For females, these traits were average egg mass, clutch size, circulating VTG and VLDL levels, and laying interval (see Williams, 1996; Salvante and Williams, 2002). For males, the traits were number of bill wipes, number of copulations, frequency and duration of song, and time of first copulation (all were repeatable in this study; for frequency and duration of song, see also Balzer and Williams, 1998). We restricted subsequent analyses to these phenotypic traits.

"High quality" females that initiated egg laying early had significantly lower nestling baseline CORT (total CORT, $F = 8.84$, $p = 0.014$; free CORT, $F = 21.90$, $p = 0.001$) and adrenocortical response (free integrated CORT, $F = 6.27$, $p = 0.031$) compared with "low quality" females that initiated egg-laying relatively late. However, average egg mass, clutch size, circulating VTG, and VLDL levels in females were all independent of any of the measured components of the adrenocortical response at both nestling and adult stage in females ($p > 0.05$). "High quality" males that produced longer and more frequent songs had significantly lower adult total and free baseline CORT compared with "low quality" males with shorter, less frequent songs (Fig. 4b, frequency: total CORT $F = 7.75$, $p = 0.018$; free CORT $F = 21.60$, $p = 0.001$; duration:
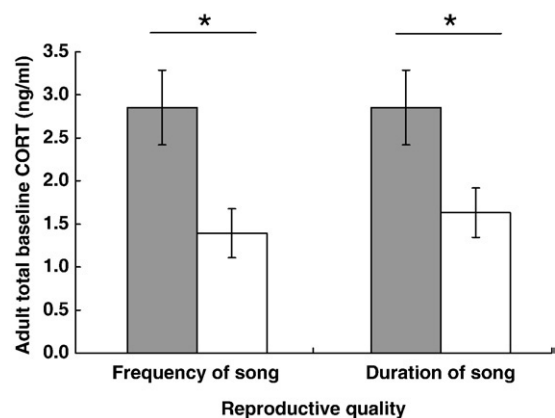

Fig. 4. Relationships between sex-specific phenotypic qualities and corticosterone levels. For both sexes, high reproductive qualities were linked with low baseline corticosterone and/or adrenocortical response. The figure represents the mean frequencies and duration of song ±1 SE in males. Filled and open bars depict low and high reproductive quality, respectively. Asterisks indicate $p < 0.05$.

total CORT $F = 5.60$, $p = 0.037$; free CORT $F = 15.03$, $p = 0.003$). However, number of bill wipes was not significantly correlated with any of the measured components of the adrenocortical response at either stage in males ($p > 0.05$).

Discussion

In this study, we found marked inter-individual variation in the adrenocortical response in both sexes and ages, e.g., total and free CORT varied as much as 29-fold and 70-fold between individuals, respectively. On average, ~ day 16 nestlings had a significantly greater adrenocortical response than adults, both for total and free CORT levels, and this difference was significant by 15 min post-handling. We found sex differences in inter-individual variation in the adrenocortical response, repeatability, and relationships between CORT levels and phenotypic quality. In male zebra finches, there was some evidence that variation in nestling adrenocortical response was systematically, but weakly, related to aspects of the nestling environment and body condition (brood size, nestling mass). However, adrenocortical response was not repeatable between nestlings and adults in males and adult phenotypic quality was independent of variation in nestling CORT. Adult phenotype (song duration and frequency), however, was significantly correlated with adult CORT. In females, the marked variation in nestling CORT was independent of aspects of nestling "environment" and body condition. However, there was evidence of repeatability of adrenocortical response between nestlings and adults, and adult phenotypic quality (timing of onset of egg laying) was correlated with variation in nestling CORT (but not adult CORT).

Age-related differences in adrenocortical response

We found that altricial zebra finches exhibited higher adrenocortical responses as nestlings than as adults. Although altricial young typically have no or low adrenocortical response during a critical period of development (stress hyporesponsive period (SHRP), reviewed in Sapolsky and Meaney, 1986; Walker et al., 2001; Vazquez, 1998; in altricial birds, Romero et al., 1998; Sims and Holberton, 2000; Wada et al., 2007), it is likely we sampled chicks after this period of development in zebra finches. Rather, the high adrenocortical response seen in our study is possibly related to the nestlings' proximity to fledging. CORT increases prior to fledging in a number of avian species including American kestrels (*Falco sparverius*) (Heath, 1997; Sockman and Schwabl, 2001), pied flycatcher (*Ficedula hypoleuca*) (Kern et al., 2001), canaries (*Serinus canaria*) (Schwabl, 1999), and Laysan Albatross (*Phoebastria immutabilis*) (Seabury Sprague and Breuner, 2005). In screech-owls, the CORT levels peak when the maximum locomotor activity for dispersal is observed (Belthoff and Dufty, 1998). In fact, CORT is shown to facilitate locomotor activity at least in adult birds (Astheimer et al., 1992; Breuner et al., 1998). In addition, CORT regulates metabolism via glucose mobilization and fat depletion (Sapolsky et al., 2000). Thus, it may be adaptive for nestlings near fledging to have higher levels of CORT than adults to help initiate the sudden increase in locomotor activity and high metabolic demands.

Individual variation in adrenocortical response

Despite a controlled environment, zebra finches in our study exhibited large inter-individual variation in HPA reactivity both as nestlings and adults. The variation seen in this study is comparable or possibly larger than previous studies that reported between an 8- and an 11-fold difference for baseline total CORT (Bourgeon et al., 2006; Guimont and Wynne-Edwards, 2006; Love et al., 2003) and between a 5- and a 15-fold difference for stress-induced CORT (Guimont and Wynne-Edwards, 2006; Schjolden et al., 2005).

Several factors have been implicated to influence individual variation in HPA axis reactivity, including genetic factors (Evans et al., 2006; Satterlee and Johnson, 1988; Tanck et al., 2001), body mass/condition (in birds: Breuner and Hahn, 2003; Heath and Duffy, 1998; Kitaysky et al., 1999a,b, 2001; Love et al., 2005; Sockman and Schwabl, 2001), sex, age, reproductive status (Bourgeon et al., 2006), and stress coping style (Koolhaas et al., 1999; Schjolden et al., 2005). In our study, we found some evidence that nestling environment such as brood size is another factor that could shape the HPA axis in avian young. It is possible that CORT increases with brood size due to higher sibling competition, food deprivation, and/or unpredictability of feeding (Kern et al., 2001; but also see Romero et al., 2006). Since a captive colony with an *ad lib* food supply was used in this study, it is unlikely that the rise in CORT is due to food deprivation or unpredictability of feeding. However, it is possible sibling competition intensifies as nestlings grow older and larger in a confined nest box. Males might be more sensitive to nestling environment than females, in terms of growth and development of the HPA axis (Love et al., 2005), which might explain why nestling CORT was independent of brood size, etc., in female nestlings. Consistent with previous studies (cited above), body mass was negatively correlated with total baseline CORT in male nestling zebra finches. Although we did not have a large enough sample size to analyze for the relationship between laying or hatching order and CORT levels, these are other possible factors influencing HPA axis in birds (Love et al., 2003).

Adrenocortical responses to a standardized stressor have been shown to be consistent within individuals in adults in some studies (Littin and Cockrem, 2001; Cockrem and Silverin, 2002; Schjolden et al., 2005, although these studies did not explicitly calculate repeatability). Here we have shown that some components of the adrenocortical response are also repeatable across ages as well, i.e., the extent to which nestlings HPA reactivity predicts adult HPA reactivity, although this repeatability is only seen in females. However, most components of HPA reactivity remained plastic between ~ D16 nestlings to adults, i.e., they were not significantly repeatable. Numerous mammalian studies show that early experiences, such as prolonged maternal separation and exposure to CORT, can result in an increased HPA reactivity in adulthood (reviewed in Caldji et al., 2001). The current study indicates that experiences during nestling development do not fix all aspects of the HPA axis in zebra finches. The lack of repeatability in this study despite the common environment indicates two points: (1) the environment during development is not the sole determinant of the HPA reactivity as adults, and (2) genetics may

play a larger role in determining individual variation in zebra finches. This points to the importance of confirming repeatability/predictability of endocrine responses if one uses developmental responses to predict future quality.

Inter-individual variation in corticosterone in relation to phenotypic quality

Chronically elevated CORT in adults inhibits reproduction in many taxa (see Sapolsky et al., 2000). More interestingly, an early exposure to CORT during embryonic or neonatal development modifies adult reproduction as well (Spencer et al., 2003). For example, CORT administration and food reduction during pre- and post-fledging have been shown to have pleiotropic effects, reducing song duration and complexity as well as growth in zebra finches. Here we have shown that natural variation in CORT is correlated with reproductive quality of sexually mature adults. In both sexes, low total baseline CORT was correlated with high reproductive quality; however, there was a sex difference in the timing of this correlation. In males, the correlation was found with adult levels, while the correlation was observed with nestling levels in females. Adult baseline CORT may indicate the individual's current conditions, such as body condition (Love et al., 2005; Pereyra and Wingfield, 2003; Romero and Wikelski, 2001) and therefore reflects how suitable they are to breed, or their resistance to infection (Hõrak et al., 2006). On the other hand, between-colony variation in baseline CORT is already apparent during a nestling period in altricial birds (Blas et al., 2005). Thus, nestling baseline CORT may provide information about the genetic makeup of their HPA axis reactivity. However, very few studies tie natural variation in acute adrenocortical response to fitness measures, such as individuals' reproductive quality, in the wild. Our study indicates that although levels of integrated total CORT did not correlate with high reproductive quality in females, levels of integrated free were related to early initiation of egg laying. This not only ties high reproductive quality with lower acute adrenocortical response but also emphasizes the importance of exploring the free CORT levels.

This is one of the few studies showing adrenocortical responses are repeatable across ages. Furthermore, it is one of the first studies linking the baseline and acute stress reactivity to an individuals' reproductive quality. With an intriguing significance of individual variation in CORT uncovered, many more studies are needed to report and explore the implications of individual variation in endocrine systems.

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