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RESEARCH ARTICLE

Are individuals consistent? Endocrine reaction norms under different ecological challenges

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ABSTRACT

Quantifying organismal capacity for compensatory mechanisms is essential to forecast responses to environmental change. Despite accumulating evidence for individual variation in physiological plasticity, the causes and consequences of this variation remain unclear. An outstanding question is whether individual reaction norms are consistent across different environmental challenges, i.e. whether an individual that is responsive to one environmental variable will be equally responsive to a different environmental variable. Additionally, are these reaction norms themselves consistent over time, i.e. repeatable? Here, we quantified individual baseline glucocorticoid responses in house sparrows, *Passer domesticus*, to sequential manipulations of temperature, wind speed and food unpredictability that were repeated in discrete blocks of sampling under both control and stressor-exposed conditions. Individuals significantly decreased their baseline corticosterone levels and increased their mass during treatment exposure. This response was consistent across environmental challenge types. There was high repeatability in the intercept and slope of the baseline corticosterone reaction norm between environmental challenges but broad credible intervals in the repeatability of the reaction norm slope, suggesting that although glucocorticoid levels during baseline conditions are repeatable, among-individual variation in the shape of the glucocorticoid response may be higher than within-individual variation. Within-subject variation in baseline corticosterone levels was mainly explained by within-individual variation in body mass during stressor exposure. Despite the high lability in physiological traits, endocrine plasticity is repeatable across environmental challenges and may be able to evolve as a result of genetic accommodation, in which selection acts on genetic variation of reaction norms.

KEY WORDS: Glucocorticoids, Corticosterone, House sparrows, Wind, Temperature, Food unpredictability

INTRODUCTION

Assessing the level of repeatability and heritability of physiological plasticity is a cornerstone to understanding the potential genetic basis of individual differences in plasticity (Scheiner and Lyman, 1989) and ultimately the role of plasticity in shaping evolutionary responses to environmental change (Diamond and Martin, 2016). There is an increasing focus on the proximate mechanisms and the ultimate factors that maintain individual differences (Gomez-Mestre and Jovani, 2013; Williams, 2008). Although physiological traits are inherently plastic, little attention has been paid to the individual differences in physiological plasticity. Individual variation in plasticity can be quantified using a reaction norm approach, in which the slope of the reaction norm documents the change in phenotypic traits as a function of an environmental gradient or time (Nussey et al., 2007; Pigliucci, 2005). Despite current interest in hormonal plasticity, there is little understanding of the causes and constraints which produce these individual differences in plastic traits (Hau and Goymann, 2015; Pigliucci, 2005; Taff and Vitousek, 2016). An important question is whether plasticity differs among individuals, i.e. some genotypes generally show greater responsiveness in a given trait to different environmental challenges than others. This situation occurs if, within each individual, there are correlations of reaction norm slopes for different environmental challenges, as a result of common proximate factors that underlie plasticity (Sih et al., 2004).

Glucocorticoids are highly conserved vertebrate hormones that mediate a suite of functional responses to changing internal and external conditions (Hau et al., 2016). Even though there is accumulating evidence that optimal glucocorticoid responses to different environments increase organismal survival and reproduction (Bonier et al., 2009; Breuner, 2011), studying the evolution of glucocorticoids is difficult (Bonier and Cox, 2020; Bonier and Martin, 2016). Most of this challenge lies in high within-individual variation in hormone secretion masking among-individual differences that selection might act upon. Thus, it is difficult to interpret selection on among-individual variation in hormone levels (but see Ouyang et al., 2013; Cox et al., 2016; Crespi et al., 2013; John-Alder et al., 2009; McGlothlin et al., 2010; Patterson et al., 2014). Many studies report on the repeatability of glucocorticoid traits in order to demonstrate stable among-individual differences (Careau et al., 2020; Grant et al., 2020). In fact, systematic reviews and meta-analyses also show high estimates of glucocorticoid repeatability in vertebrates (Schoenemann and Bonier, 2018; Taff et al., 2018a). The ability of an organism to repeatedly adjust its phenotype under changing environmental conditions should be critical to maximize fitness, but we still understand little about whether and how individuals differ in their glucocorticoid plasticity (but see Guindre-Parker et al., 2019; Houslay et al., 2019; Lendvai et al., 2014; Sonnweber et al., 2018). Understanding the degree to which individuals differ in their glucocorticoid plasticity will impact our ability to predict organismal responses to environmental change and ultimately how selection may act on glucocorticoids (Angelier and Wingfield, 2013; Guindre-Parker, 2020).

Researchers have quantified among-individual covariance between reaction norm slopes of the stress response to different environmental gradients, but plasticity has been a secondary focus (Careau et al., 2020; Guindre-Parker et al., 2019; Lendvai et al., 2015), as environmental gradients were varied simultaneously. Therefore, the question of whether glucocorticoid plasticity is consistent (repeatable) for each individual across different

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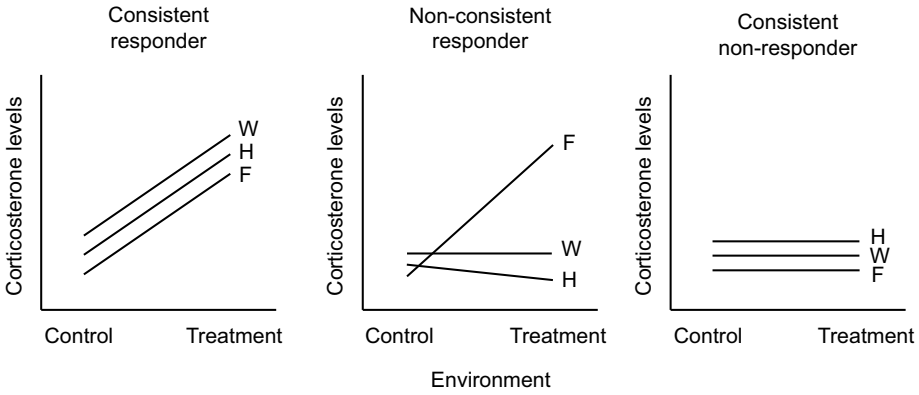

Fig. 1. Hypothetical illustration of corticosterone reaction norms (slope) under control and treatment conditions. A consistent responder is an individual that elevates glucocorticoid levels irrespective of environmental challenge type: here, wind (W), heat (H) and food predictability (F). A non-consistent responder is an individual whose reaction norms differ in response to environmental variable type. A consistent non-responder is an individual that does not respond to any of the environmental challenges.

challenges, i.e. whether individuals respond in the same way to changes in different environmental variables, remains an open question. Individuals may be consistent in their degree of flexibility (some individuals always respond in the same manner whereas others might not respond to any environmental challenge), which may be the result of the same underlying physiological mechanisms (Fig. 1). However, the level of plasticity of each individual may differ across different challenges, and individuals may express different reaction norms across time or contexts (Araya-Ajoy and Dingemanse, 2017). Addressing this question is particularly interesting as consistency of individual differences in reaction norm slopes is an underlying assumption for the evolution of physiological plasticity, because it is a prerequisite for heritable variation. However, we have limited evidence of the repeatability of glucocorticoid plasticity (Lendvai et al., 2014; Sonnweber et al., 2018) especially in response to different environmental challenges.

Investigating repeatability of glucocorticoid plasticity requires information on within- and among-individual variation in glucocorticoid responses. In this study, we explored variation in the baseline glucocorticoid secretion of house sparrows, *Passer domesticus*, in response to three different yet ecologically relevant environmental challenges (high temperature, high wind and unpredictable food resources). By exposing each individual to each environmental challenge in a random block design, we were able to partition the within-individual variation in plasticity, i.e. how each individual differs in its response to the three challenges, and the among-individual variation in plasticity, i.e. how different individuals differ in their response to the three challenges, to calculate repeatability of the glucocorticoid response. Specifically, in this study we tested whether individuals are (1) on average plastic and (2) consistent in the direction and magnitude of the baseline endocrine response across different challenges. Firstly, individuals may or may not respond to environmental challenges, and thus can be categorized into responders or non-responders (Fig. 1). If they are responsive, they can be either consistent or non-consistent in their response depending on whether the reaction norm slope on average differs within each individual across the different challenges (Fig. 1). This is a 'population'-level description of physiological plasticity as it depends on the average response of all individuals to the different challenges.

Secondly, reaction norms of glucocorticoids to different environmental challenges under control and treatment conditions can be repeatable (i.e. low within-individual variation) or non-repeatable (i.e. high within-individual variation; Fig. 2). High repeatability in glucocorticoid reaction norm would occur if each individual responds in the same way (similar slope) to different challenges, such that among-individual variation is higher than within-individual variation (Fig. 2). Low repeatability would instead occur if each individual responds differently to different challenges such that the among-individual variation is lower than within-individual variation (Fig. 2). We expected all individuals to increase their baseline corticosterone levels following a period of environmental challenge exposure, and that this response would be consistent across challenges and repeatable among individuals. Because in a previous study we found that an increase in corticosterone levels as a result of food restriction was associated with a decrease in body mass (Lendvai et al., 2014), we also investigated the effect of treatment exposure on body mass and its relationship with corticosterone changes. In this case, we expected a negative relationship between corticosterone levels and body mass, with the latter decreasing as corticosterone increases during the treatment phase.

MATERIALS AND METHODS

Study subjects

For this experiment, 12 male adult house sparrows, *Passer domesticus* (Linnaeus 1758), were randomly chosen from a captive population of sparrows housed in outdoor aviaries at the University of Nevada, Reno (captured as fledglings from the wild in autumn 2016). In 2019, these 12 experimental individuals were moved into individual cages (47×31×36 cm) in an indoor facility on a 12 h:12 h light:dark photoperiod with access to *ad libitum* water and food. Upon capture, tarsus length (to the nearest 0.1 mm) and body mass (to the nearest 0.25 g) were measured. The laboratory room was set with a constant temperature of 21°C, constant humidity of 30% and a constant airflow of 0.01 $m^3\ s^{-1}$ per bird. Individuals were visually isolated with opaque barriers and acoustically isolated via white noise played constantly during the habituation and the experimental period from a small speaker. All procedures were approved by the University of Nevada, Reno, Institutional Animal Care and Use Committee and performed in accordance with NIH guidelines.

Experimental protocol

Upon release into the experimental cages, birds were given a 2 week habituation period, in which water and food were provided *ad libitum*. After the habituation period, each individual underwent a 12 week experimental period in which they were consecutively exposed to three different environmental challenges (heat exposure, wind exposure and unpredictable food availability; see Fig. S1). The experimental period was divided into three 4 week sessions, in which one environmental challenge was applied singularly for 4 weeks. The temporal order of the environmental challenge exposure was randomized across individuals, and each bird was

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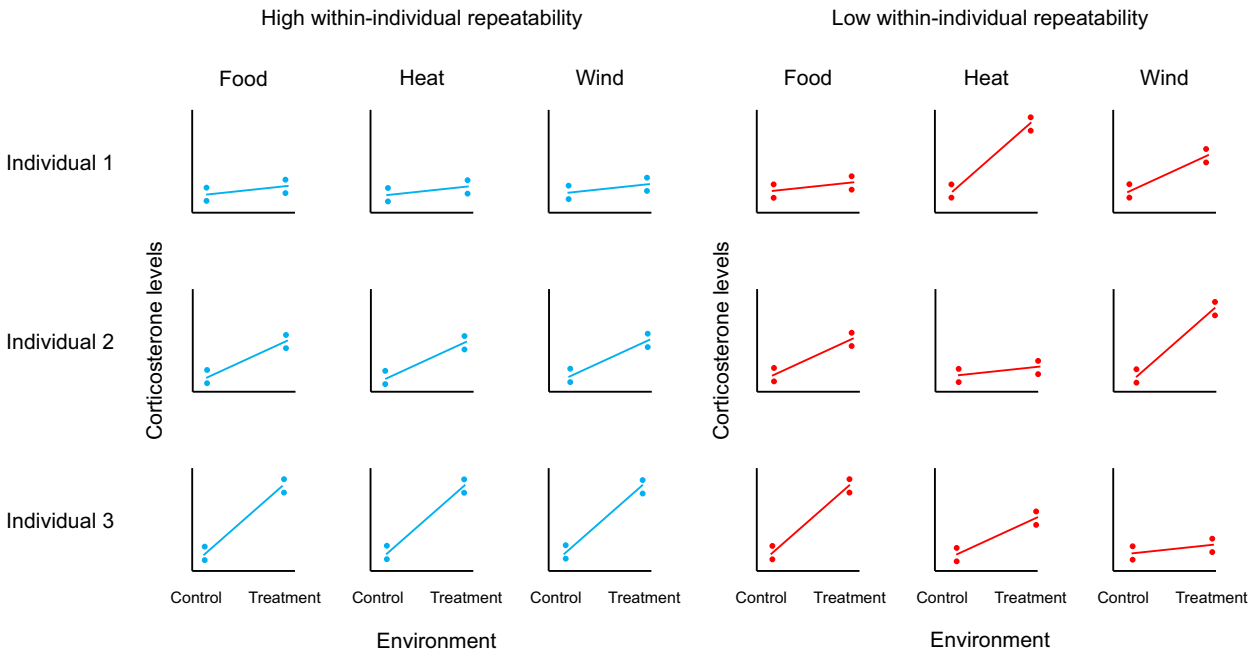

Fig. 2. Hypothetical illustration of the corticosterone reaction norms under control and treatment conditions, showing within- and among-individual plasticity. A situation of high repeatability would occur if each individual responded in the same way to different environmental challenges, such that among-individual variation is higher than within-individual variation. A situation of low repeatability would occur if each individual responded differently (in the magnitude of the response but not in the direction) to different environmental challenges, such that among-individual variation is lower than within-individual variation. Note that both scenarios would have no significant interaction between environmental challenge type and treatment (control or treatment phase), as the mean slope for each environmental challenge is the same across environments. In other words, these two scenarios apply in the situation in which individuals acted on average as consistent responders. Scenarios can occur in which there is low consistency in the response but high among-individual variation in the slope of the response, resulting in repeatability of the slope (not illustrated).

exposed to all three environmental challenges. This set up allowed a complete within-individual comparison and controlled for possible carry-over effects of environmental exposure.

For each environmental challenge, the 4 week session was organized into two control weeks (week one and three), in which the environmental challenge was not applied, and two treatment weeks (week two and four), in which birds were exposed to the environmental challenge. The control–treatment phases were repeated twice for each environmental challenge (e.g. repeat one: week one and two; repeat two: week three and four) to better estimate the individual reaction norms, which are based on four data points (see Fig. 4). The heat treatment consisted of a 75 W heat lamp (14 cm of diameter) placed ca. 10 cm from the front side of the cage, which increased the temperature inside the cage to 29°C. The wind treatment consisted of a mini electric fan (13 cm diameter) placed ca. 10 cm from the front side of the cage. When active, the fan produced an 8 m s^{-1} wind current inside the cage. House sparrows were caught in environments in which natural daily temperatures range from 7.8 to 33.8°C and average wind speeds range from 2.7 to 10 m s^{-1} (National Weather Service, https://www.weather.gov/). The heat lamp and fan were turned on 24 h per day during the treatment weeks, whereas they were present but inoperative outside the cage during the control weeks. During the heat and wind exposure sessions, water and food were provided *ad libitum* during both the control and treatment weeks. Unpredictable food availability was created by removing the food tray (the bottom of the cage was also carefully cleaned so that no food was present inside the cage) for six consecutive hours during the light period. The 6 h food removal started randomly between 08:00 h and 14:00 h (and therefore the final period lasted between 14:00 h and 20:00 h) and varied randomly every day during the treatment weeks. Food was available *ad libitum* during the control weeks.

Blood samples and body mass were collected from each bird at 08:00 h at the end of each week. All blood samples were taken within 3 min from the time we entered the room (2.4±0.08 min, mean±s.e.m.). Samples were spun for 10 min at 16,000 ***g*** within 30 min of collection. Plasma was immediately separated and frozen at −20°C until analysis.

Hormone assay

To measure plasma corticosterone, we used an enzyme-linked immunosorbent assay kit (Enzo Life Sciences, Farmingdale, NY, USA) following the manufacturer's instructions, with a standard curve on each plate. To validate this assay for use with house sparrow plasma, we first removed endogenous hormones from the plasma by incubating it for 20 min in a solution of 1% charcoal and 0.1% dextran. We then added sufficient corticosterone standard from the assay kit so that the concentration of corticosterone in each stripped plasma sample was equal to 500 pg ml^{-1}. We assayed each stripped and spiked sample at three dilutions (1:20, 1:30 and 1:40) and each dilution with two concentrations of steroid displacement reagent (SDR; 0.5% and 1% of plasma volume). Based on this optimization, we determined that for subsequent assays, house sparrow plasma should be diluted 1:40 with 0.5% SDR. We randomly assigned samples across four plates, with the exception that all samples from the same individual were on the same plate. We included a standard curve on each plate, which ranged from 32 pg ml^{-1} to 20,000 pg ml^{-1}. The assay sensitivity was 2.1 pg ml^{-1}. To calculate intra- and inter-plate coefficient of variation (CV), we also included three pooled house sparrow samples on each plate, and each pool was assayed in triplicate. The intra-plate CV was 7.9% and inter-plate CV was 3.9%.

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Statistical analysis

We performed all the statistical analysis in the R environment version 3.2.3 (http://www.R-project.org/). We ran all linear mixed models (LMMs) with the lmer function in the lme4 package (Bates et al., 2015). All final models met assumptions of normality and homoscedasticity of residual errors, and significance was taken at α=0.05. To provide further support on the outcomes of the mixed models, we also performed a corrected Akaike information criterion (AICc) comparison (Anderson and Burnham, 2002) of all models. For Markov chain Monte Carlo mixed models, we report the parameter estimates and the corresponding 95% credible intervals in brackets, i.e. mean [lower 95% CI, upper 95% CI].

First, we investigated the effect of the three environmental challenges on corticosterone levels and body mass. We fitted LMMs with 'corticosterone' and 'mass' as the response variable, 'environmental challenge' (three level variable: heat exposure, wind exposure and unpredictable food availability) and 'treatment' (two level variable: control and treatment phase) as fixed effects. Because we were interested in whether the individual corticosterone reaction norm (the difference between control and treatment) differs across environmental challenges, we also tested for the interaction between 'environmental challenge' and 'treatment'. In these models, we also included 'date' as covariate, 'Individual ID' and 'RepeatID' nested within 'challengeID' as random effects to account for repeated measures and the structure of the experimental set up. In these analyses, we log transformed corticosterone concentrations to meet assumptions of normality of model residuals. For the analyses of corticosterone levels, we also carried out an AICc comparison between the model with and without 'individual ID' as random intercept to investigate whether individual variation in the baseline (intercept) corticosterone levels better explained our dataset.

Second, we estimated the level of repeatability of the slope and intercept of the baseline corticosterone response during the three environmental challenge changes using a mixed-model reaction norm approach following Araya-Ajoy et al. (2015). This random regression approach enables the population average response of the trait of interest (intercept and slope), via the fixed effect component of the model, and the individual phenotypic deviations from the population average intercepts and slope, via the random effect structure ('Individual ID') to be distinguished (Nussey et al., 2007). Typically, this method estimates a single reaction norm intercept and slope for each individual. As we also wanted to quantify within-individual variation in intercepts and slopes, in addition to 'Individual ID', we included an additional random effect of 'Series' (a series is the equivalent of one 4 week session, three series per individual; see Fig. S1) as described in Araya-Ajoy et al. (2015). In this way, the multi-level random regression mixed-effect models quantify the variation in reaction norm intercepts and slopes within and among individuals (Araya-Ajoy et al., 2015). In this analysis, we only included individuals with three completed series (n=11, series=33). The log-transformed corticosterone concentrations were modeled as a function of treatment exposure (control versus treatment phase) and environmental challenge type, which was fitted as an environmental covariate with three levels (heat exposure, wind exposure and unpredictable food availability). Random intercepts were included for individual and series; random slopes with respect to treatment exposure were also included at two hierarchical levels. We fitted the random regression model using a Bayesian framework implemented with the package MCMCglmm (Hadfield, 2010). We ran 3,003,000 iterations per model, from which we discarded the initial 3000 (burn-in period). Each chain was sampled at an interval of 3000 iterations. Repeatabilities of slope and intercept were calculated as in Araya-Ajoy et al. (2015) as follows: intercept repeatability was calculated as the amount of among-individual variance in intercepts divided by the total variance in intercepts (i.e. the sum of the among-individual and among-series variances in intercept); slope repeatability was estimated as the amount of among-individual variance in slopes divided by the total phenotypic variation in slopes (i.e. the sum of the among-individual and among-series variances in slope). Posterior means and 95% CIs were estimated across the thinned samples for the mean effects (fixed effects), (co)variances and repeatabilities. We deemed the slope and intercept of the corticosterone response as repeatable if the CIs did not overlap zero.

Lastly, we explored whether the change in corticosterone levels during the experiment was associated with changes in body mass. We fitted a LMM with 'corticosterone' as response variable, 'mass' as fixed effect and 'Individual ID' as random term. To understand whether the relationship between corticosterone and body mass was due to a within-subject effect (e.g. corticosterone changes in conjunction with changes in body mass in each individual) and not a between-subject relationship (e.g. heavier birds may have lower or higher corticosterone levels than low mass birds), we used the within-subject centering method (van de Pol and Wright, 2009). Briefly, for each individual we calculated the mean body mass (between-individual variance component) and the difference from its own mean (within-individual variance component) and fitted these two variables as predictors in a LMM with 'corticosterone' as response variable. In this model, corticosterone was log transformed to improve normality of model residuals.

RESULTS

Corticosterone levels differed between the control and treatment phase: on average individuals significantly decreased their corticosterone levels (17% decrease compared with control) during the environmental challenge exposure (Figs 3A and 4 for individual reaction norms). There was no significant interaction between environmental challenge and treatment, indicating that on average, the slope in corticosterone response did not vary across the

Table 1. Model estimates for the effects of environmental challenge type and treatment exposure on $\log_{10}$-transformed corticosterone levels and body mass

Variable	Estimate	s.e.	t	P
LMMs for corticosterone levels (R^2=0.50)				
(Intercept)	−10.32	49.36	−0.21	0.834
Heat (reference Food unpredictability)	−0.27	0.19	−1.39	0.162
Wind	−0.29	0.20	−1.48	0.139
Treatment	−0.38	0.15	−2.47	**0.014**
Date	0.00	0.00	0.22	0.824
Heat×Treatment	0.26	0.21	1.23	0.218
Wind×Treatment	0.05	0.21	0.22	0.828
LMMs for body mass (R^2=0.56)				
(Intercept)	219.83	65.43	3.35	**<0.001**
Heat (reference Food unpredictability)	−0.06	0.30	−0.22	0.825
Wind	0.41	0.30	1.38	0.168
Treatment	0.70	0.30	2.32	**0.020**
Date	−0.01	0.00	−2.97	**0.003**
Heat×Treatment	−0.65	0.41	−1.55	0.120
Wind×Treatment	−0.28	0.42	−0.67	0.503

Individual estimates are given from the summary statistics of the linear mixed models (LMMs). 'Individual ID' and 'RepeatID' nested in 'challenge ID' were included as random effects in the models. Significant terms are in bold.

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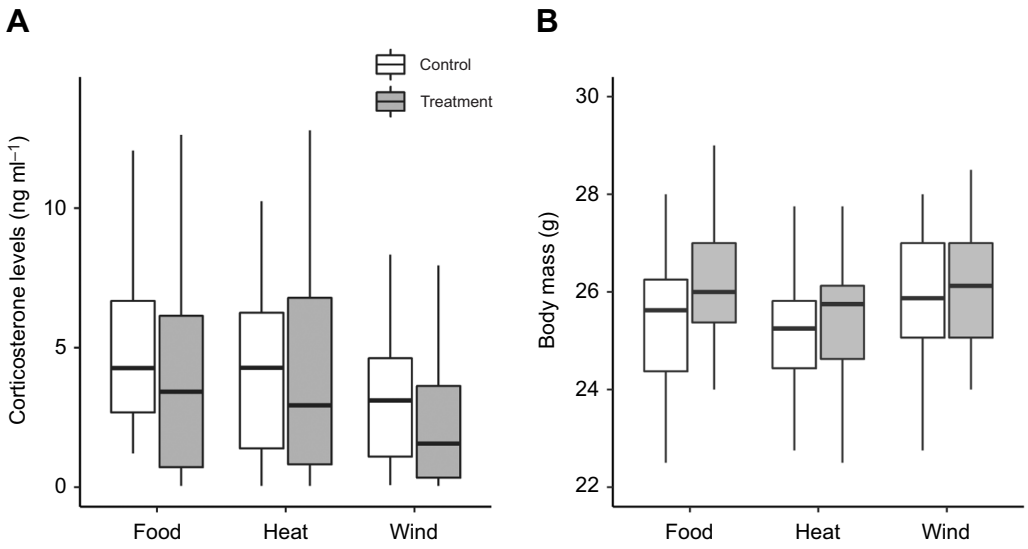

Fig. 3. Boxplots of corticosterone levels and body mass across the three challenge types during the control and treatment phase. Box plots show median (horizontal line inside boxes), upper and lower quartiles (box) and 1.5× interquartile range (whiskers) for (A) corticosterone levels and (B) body mass.

different challenges (Table 1). Therefore, in general, individuals were consistent responders (Fig. 1). Furthermore, the corticosterone model with 'Individual ID' as random term had a better fit than the model without the random term (ΔAIC between the model with and without the random effect=3.75, see Table S1) indicating that there was individual variation in the baseline (intercept) corticosterone levels. Initial body mass significantly differed between environmental challenge types and increased during exposure to the stressor (Fig. 3B, Table 1). Similar to corticosterone levels, there was no significant interaction between environmental challenge and treatment, suggesting that the increase in body mass did not vary across the different environments. Body mass also slightly decreased throughout the experimental period (−0.010 ±0.003 g day^{-1}, Table 1). AICc model comparisons supported the results of the LMMs for corticosterone levels and body mass (Table 2).

The reaction norm analysis found evidence for repeatability in intercept (R=0.63 [0.23, 1.00]; Table 2) and slope (R=0.61 [0.02, 1.00]; Table 3) of the corticosterone reaction norm between environmental challenges, as their CIs did not statistically overlap with zero. However, for the repeatability of the reaction norm slope, we found broad CIs, suggesting low confidence in slope repeatability. Overall, these results suggest that individuals had a repeatable corticosterone response to external stimuli (Figs 2 and 4 for individual reaction norms).

Throughout the experiment, corticosterone levels were negatively associated with individuals' body mass ($F_{1,116}$=32.42, P<0.001): on average, individuals reduced their circulating corticosterone levels by 0.98±0.24 ng ml^{-1} for every 1 g of mass gained (Fig. 5). The within-subject centering analysis showed that the variation in corticosterone levels was mainly explained by within-individual variation in body mass, while the between-individual effect was weaker (Table 4, Fig. 6).

DISCUSSION

Consistency in the direction and magnitude of plastic responses across environmental challenges is often presumed, but we lack empirical evidence for this variation in labile physiological traits. We tested within- and among-individual variation in baseline corticosterone response to different environmental challenges and showed that, in general, individuals behaved as consistent responders, decreasing their baseline corticosterone levels and increasing their body mass in response to different external stimuli. There was also repeatability in slope and intercept of the baseline corticosterone response, suggesting individual differences in the physiological response across environmental challenges.

Table 2. Comparative fit of the top five models for corticosterone levels and body mass in relation to environmental variable type and treatment exposure

Variable	Rank	Model	d.f.	logLik	AICc	ΔAICc
Corticosterone levels						
	1	Treatment	6	−126.64	260.49	0.00
	2	Treatment+environmental challenge	8	−127.26	262.27	1.78
	3	Treatment+Date	7	−131.62	262.68	2.19
	4	Treatment+environmental challenge+Date	9	−132.23	264.57	4.08
	5	Treatment+environmental challenge+Treatment×environmental challenge	10	−127.78	265.05	4.59
Body mass						
	1	Treatment+environmental challenge+Date	9	−212.58	430.09	0.00
	2	Treatment+environmental challenge+Date+Treatment×environmental challenge	11	−211.41	432.24	2.15
	3	Environmental challenge+Date	8	−214.13	432.70	2.61
	4	Treatment+Date	7	−215.65	434.86	4.78
	5	Treatment+environmental challenge	8	−211.41	435.60	5.51

d.f. is the number of parameters in the model, LogLik is the log-likelihood of the model, AICc is the Akaike's information criterion corrected for small sample sizes, whereas ΔAICc is the difference in the Akaike's information criterion between the model of interest and the most parsimonious model of the model set.

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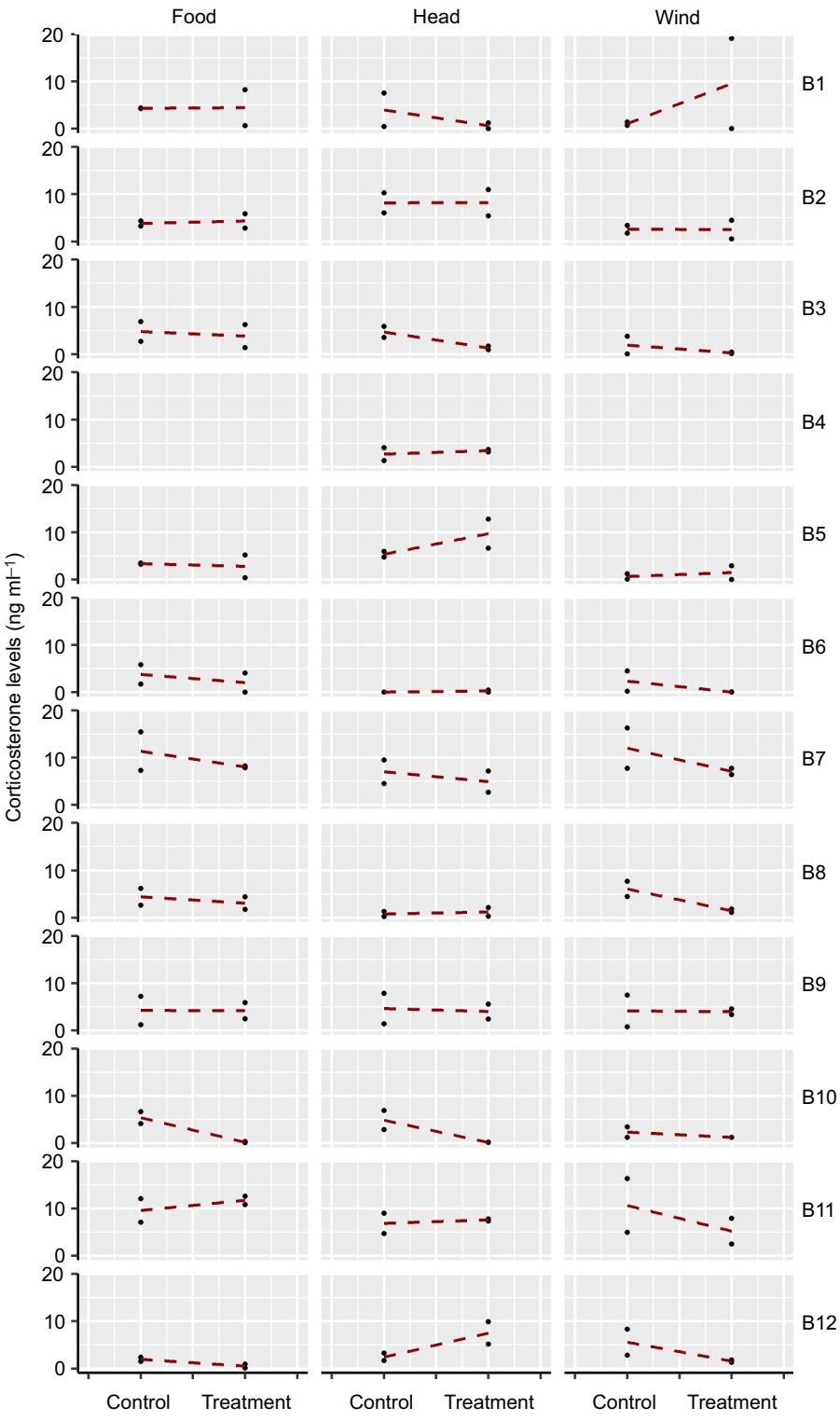

Fig. 4. Individual reaction norms of the baseline corticosterone levels in relation to the three environmental challenges. Each row shows the reaction norm of a single individual (e.g. B1) in the three environmental challenge exposures (food unpredictability, heat and wind exposure).

We found significant decreases in baseline corticosterone levels in response to all three environmental challenges. This finding contrasts with our original prediction that treatment exposure should increase circulating levels of glucocorticoids. For example, exposure to rain and cold wind in captive, non-molting starlings (*Sturnus vulgaris*) raised corticosterone levels (de Bruijn and Romero, 2013), and food restriction in house sparrows decreased mass and increased corticosterone levels (Lendvai et al., 2014). One explanation for why individuals decreased rather than increased their corticosterone response is that 1 week of continuous treatment could be perceived as a chronic stressor, which has been shown to decrease corticosterone levels (Cyr and Romero, 2007). In the chronic stress protocol applied to free-living and captive starlings, individuals were exposed to a series of acute stressors for 8–20 consecutive days and corticosterone levels decreased after stress exposure (Cyr and Romero, 2007). Therefore, although the environmental variables applied were

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Table 3. Variation in corticosterone concentration as a function of environmental challenge type and treatment exposure

	Model output		β [95% CI] or σ^2 [95% CI]	*P*
Fixed effects				
	Intercept		0.44 [0.09, 0.75]	**0.014**
	Environmental challenge *(Heat)*		−0.15 [−0.50, 0.17]	0.348
	Environmental challenge *(Wind)*		−0.27 [−0.62, 0.06]	0.118
	Treatment		−0.28 [−0.52, −0.04]	**0.016**
Random effects				
	Among individuals	V_{ind0}	0.18 [0.001, 0.43]	
		V_{ind1}	0.07 [0.001, 0.25]	
		$Cov_{ind0,\ ind1}$	0.04 [−0.06, 0.17]	
	Within individuals among series	$V_{series0}$	0.10 [0.001, 0.22]	
		$V_{series1}$	0.04 [0.001, 0.13]	
		$Cov_{series0,\ series1}$	0.01 [−0.05, 0.07]	
	Residuals		0.28 [0.20, 0.36]	
	Repeatability	*Intercept*	0.63 [0.23, 1.00]	
		Slope	0.61 [0.02, 1.00]	

We used a MCMCglmm with random intercepts and slopes (with respect to treatment and environmental variable) at the level of the individual and series within individual. Random effects representing among-individual ('ind') and among-series ('series') variance (σ^2) in intercepts have the subscript '0', and for variance in slopes they have the subscript '1'; intercept–slope covariances ('Cov') are presented at each level. The reference level for environmental challenge is unpredictable food availability. All values are reported as means with 95% credible interval (CI). Significant terms are in bold.

within the range house sparrows experience in nature, a week of continuous application may be perceived as a chronic stressor that decreased hypothalamic–pituitary–adrenal (HPA) axis activity and glucocorticoid receptor density (Dickens et al., 2009). Additionally, captive and wild animals differ in their stress response (Calisi and Bentley, 2009; DuRant et al., 2020). Our sparrows had been living in captivity for a couple of years and their stress response may be altered, but they do still show a robust stress response to capture/restraint stress (Ouyang et al., 2021). In this regard, further constant stressor exposure studies on repeatability in natural settings, e.g. constant simulation of predator presence in a foraging site or unpredictable food availability at feeders in winter for resident species, could shed light on the generality of our findings.

Table 4. Within-subject centering analysis on the relationship between corticosterone levels and body mass

qVariable	Estimate	s.e.	*F*	d.f.	*P*
Within-subject effect	−0.24	0.05	29.60	1122	**<0.001**
Between-subject effect	0.01	0.00	6.19	1,11	**0.03**

F, d.f. and *P* values are given from the removal of a term from the full model. Estimates and s.e. are given for a model containing only significant terms (shown in bold). 'Individual ID' was included as random effect in the model.

The sparrows also increased body mass in response to treatment exposure. This result is in line with a food deprivation experiment in house sparrows (Lendvai et al., 2014), in which a negative relationship was shown between changes in corticosterone and body mass, i.e. corticosterone decreased as body mass increased. One possible explanation could be that increases in corticosterone facilitate anabolic catabolism, resulting in the observed negative correlation between corticosterone and body mass (Wikelski et al., 1999). However, the repeated measures design allowed us to show that this relationship is caused by within-individual effects (van Noordwijk and de Jong, 1986). Therefore, changes in labile traits may account for most of the phenotypic variation in a population. Without recognizing within-individual variation, among-individual correlations with fitness might actually be due to within-individual changes responding to varying environmental conditions (Brommer, 2013).

We found repeatability of the intercept and slope of the reaction, so we can conclude that, on average, individuals respond physiologically to the prolonged environmental challenges with a decrease in baseline corticosterone levels and that there is significant individual variation in their response (Cockrem, 2013). These findings corroborate those of a previous study which found substantial individual differences in the corticosterone response to a food restriction experiment, with some individuals being highly responsive and others being unaffected by the manipulation (Lendvai et al., 2014). However, initial corticosterone levels, as represented by the intercept, were highly repeatable, suggesting that baseline levels of corticosterone were repeatable on a short time scale (Ouyang et al., 2011; Taff et al., 2018a). Furthermore, the existence of repeatability for the slope of the reaction norm suggests that selection may be able to act on how flexible an individual is in

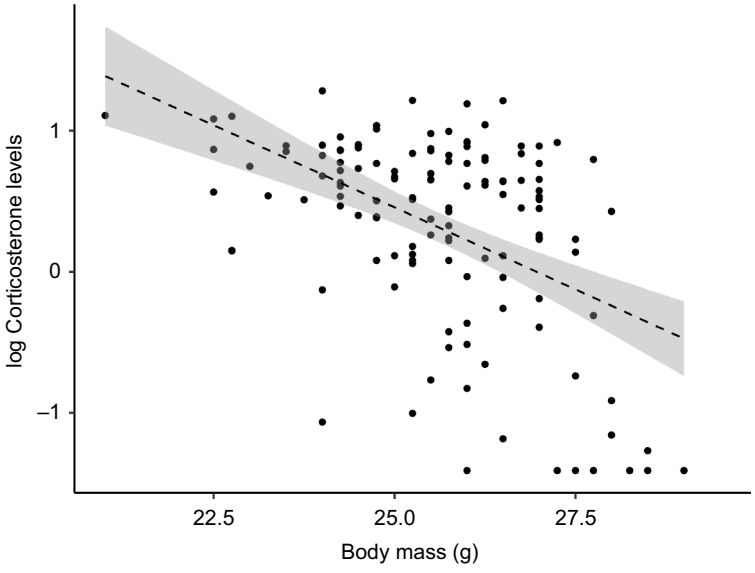

Fig. 5. Relationship between log_{10}-transformed corticosterone levels and body mass. The dashed line represents fitted values; the shaded area is the s.e. Note that individuals are represented with multiple points. The line represents the model fit of the mixed model that accounts for the repeated nature of the data.

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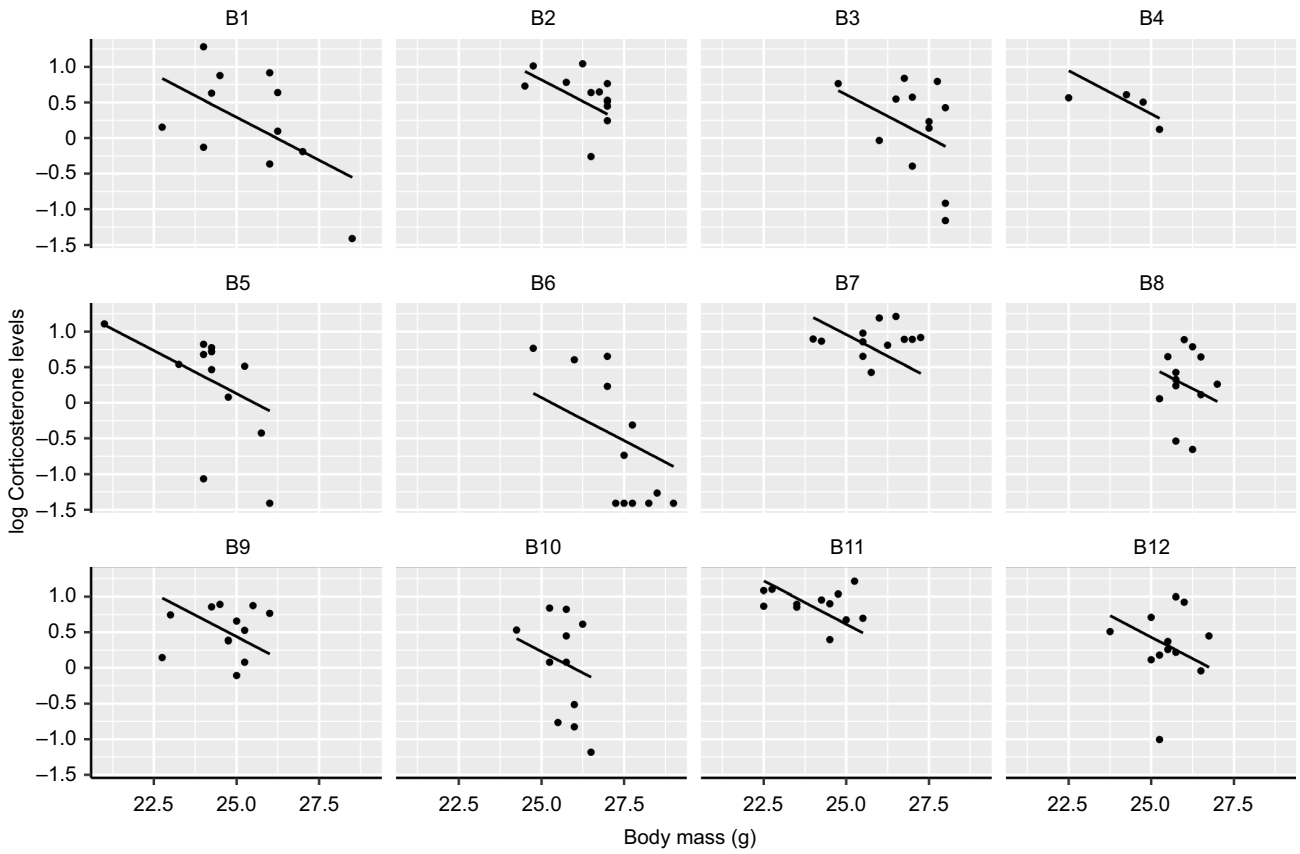

Fig. 6. Within-individual relationship of log_{10}-transformed baseline corticosterone levels in relation to body mass. Each panel represents an individual (e.g. B1). Predicted lines represent the model fit, structured with common slopes but different intercepts.

responding to environmental change. Although the slope was statistically repeatable, we note that we found large CIs in the repeatability of the slope. This could arise from several features of our experimental set-up, such as a relatively small sample size and short exposure and intensity of the stressors. In addition, there are other ways to look at the glucocorticoid response and consistency, such as using profile repeatability (Grant et al., 2020; Reed et al., 2019) or calculating the area under the curve (Delehanty and Boonstra, 2011), especially for studies that have measured hormone levels over multiple time points. These methods along with reaction norm approaches may be useful to gauge negative feedback and resilience to stress (Zimmer et al., 2019), which is an important aspect of the stress response and animal health (Dickens et al., 2010; Taff et al., 2018b).

Behavioral ecologists have often assumed that degree of flexibility is correlated across contexts, but this assumption lacks empirical support (Stamps, 2016). A recent empirical study on zebrafish showed that although there were repeatable behavioral reaction norm slopes in response to temperature and food availability, individuals that were more thermally responsive were not more responsive to food deprivation (Mitchell and Biro, 2017). Differences between behavioral and physiological plasticity may be a result of proximate causes, such as constraints on the range of available glucocorticoid receptors (Lattin et al., 2012), limiting the potential range of responses.

Despite the high lability of hormone concentrations, we have demonstrated repeatability of reaction norm components. Individuals were consistent responders (Fig. 1) with repeatable intercepts and moderate repeatability in slope (Fig. 2). The existence of multiple glucocorticoid reaction norms has been shown in a few studies in a range of vertebrate taxa (Fürtbauer et al., 2015; Guindre-Parker et al., 2019; Houslay et al., 2019; Sonnweber et al., 2018), as within-individual slopes vary in response to differences in external variables, such as population density, time of day and personality. A study in chimpanzees also showed significant repeatability over 8 years of sampling in reaction norms of fecal cortisol levels (Sonnweber et al., 2018). These studies and ours represent an important first step to understanding endocrine plasticity evolution, as repeatability is a prerequisite for heritability, which in turn informs on the evolvability of endocrine traits.

Phenotypic plasticity can facilitate or constrain evolutionary responses (Ghalambor et al., 2007). For example, if individuals do not experience different environments during their lifetime, different genotypes produce the same phenotype, and selection cannot happen because all individuals are at reaction norm intersections (Pigliucci, 2005), i.e. all individuals respond the same way to different stressors. However, a theoretical model suggests that in fluctuating environments, plasticity is favored by selection and maintained in the population (Gomez-Mestre and Jovani, 2013). Therefore, the variation and repeatability of hormone plasticity that we observed in addition to the above-mentioned empirical work across taxa suggests that selection may be able to act on this trait, especially in rapidly changing environments. Moreover, physiological plasticity can constrain evolution if it confers higher mean fitness, weakening subsequent selection by hiding genotypic variation. If all individuals have plastic responses to stressors and more plastic individuals always have higher fitness, selection on glucocorticoid expression may be difficult. However,

maladaptive plasticity can drive a population to extinction through homeostatic failure. Physiological plasticity can facilitate evolution by buffering populations from extirpation so that selection can act on standing or cryptic genetic variation. A rapid endocrine response in dynamic environments may be required to optimize organismal fitness (Ouyang et al., 2015; Vitousek et al., 2014). Although we found repeatability in reaction norm components, there was large individual variation in responsiveness. Further studies on corticosterone plasticity across multiple generations with pedigree information are necessary to investigate the heritability of physiological plasticity. In this way, we can gain insight into how physiological traits can evolve as a result of genetic accommodation, i.e. heritable changes that occur in response to a novel induction, with increased or decreased environmental sensitivity.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
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Data availability
Raw data supporting this manuscript are available from Zenodo: https://zenodo.org/record/4903342#.YLuCP_kzY2x.

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