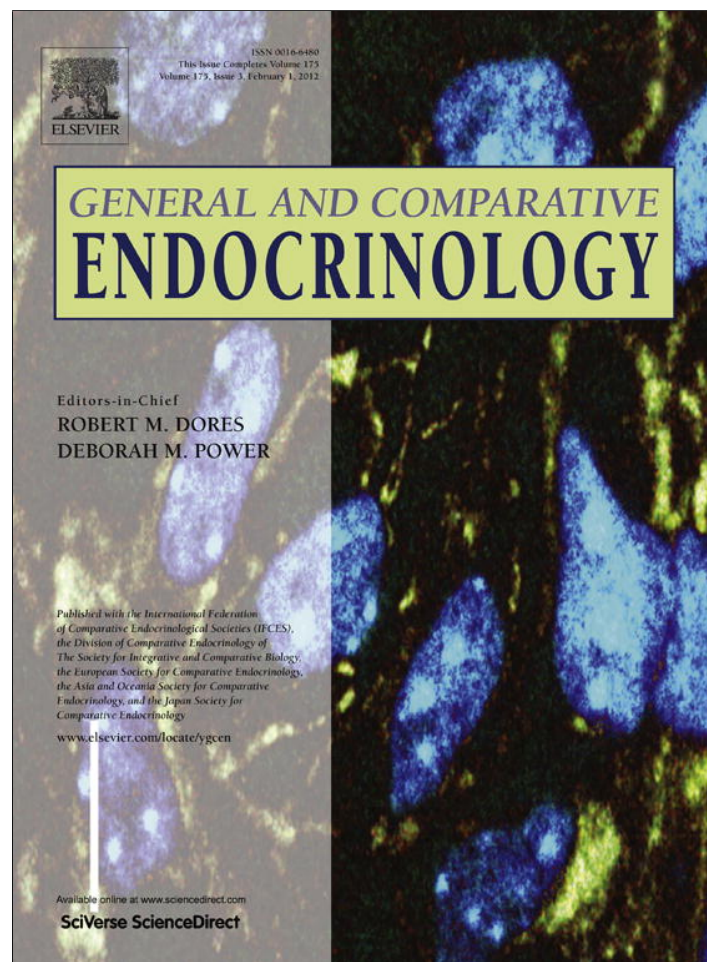


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## General and Comparative Endocrinology

journal homepage: [www.elsevier.com/locate/ygcen](http://www.elsevier.com/locate/ygcen)Corticosterone responses differ between lines of great tits (*Parus major*) selected for divergent personalitiesAlexander T. Baugh<sup>a,\*</sup>, Sonja V. Schaper<sup>b</sup>, Michaela Hau<sup>a</sup>, John F. Cockrem<sup>c</sup>, Piet de Goede<sup>b</sup>, Kees van Oers<sup>b</sup><sup>a</sup> Department of Migration and Immuno-ecology, Max Planck Institute for Ornithology, Schlossallee 2, 78315 Radolfzell, Germany<sup>b</sup> Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands<sup>c</sup> Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand

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## ABSTRACT

Animal 'personality' describes consistent individual differences in suites of behaviors, a phenomenon exhibited in diverse animal taxa and shown to be under natural and sexual selection. It has been suggested that variation in personality reflects underlying physiological variation; however there is limited empirical evidence to test this hypothesis in wild animals. The hypothalamic–pituitary–adrenal axis is hypothesized to play a central role in personality variation. Here we tested whether in great tits *Parus major* variation in personality traits is related to plasma concentrations of corticosterone (CORT). Using a capture-restraint protocol we examined baseline and stress-induced CORT levels in two captive experimental groups: (1) birds selected for divergent personalities ('fast-bold' and 'slow-shy' explorers); and (2) non-selected offspring of wild parents. We first tested for differences in CORT between selection lines, and second examined the relationship between responses in a canonical personality test and CORT concentrations in non-selected birds. We found support for our prediction that the slow-shy line would exhibit a higher acute stress response than the fast-bold line, indicating a genetic correlation between exploratory behavior and stress physiology. We did not, however, find that continuous variation in exploratory behavior co-varies with CORT concentrations in non-selected birds. While our results provide support for the idea that personality emerges as a result of correlated selection on behavior and underlying physiological mechanisms, they also indicate that this link may be particularly evident when composite personality traits are the target of selection.

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## 1. Introduction

When exposed to the same environmental challenges, individuals of a given species or population often exhibit consistent individual differences in behavioral responses (for review, see [1]). Consistency for a given behavior often correlates with other behavioral responses in different contexts and can have a heritable component (reviewed in [54]). Personality can be defined as the suite of behavioral tendencies in an individual that exhibit consistency across time and context. The study of animal personalities (also referred to as 'coping styles', 'behavioral syndromes' and 'animal temperament') aims to understand how behavioral traits co-vary within individuals and which evolutionary processes might gener-

ate such trait constellations (reviewed in: [38,48]). Indeed, a growing number of studies have now shown that personality traits can be under natural [14,15,49] and sexual selection [47,55].

A suite of neural and hormonal adaptations underlie behavioral responses to environmental and social challenges. Recently, interest in the physiological correlates of animal personality has been growing and several studies have shown that physiological responses can also be individually consistent [11,39,42,60] and related to personality (reviewed in: [27,61]). Hormones in particular, due to their systemic nature and simultaneous effects on multiple traits, have been proposed to represent key physiological mechanisms that organize behavioral traits into suites [7,27,30]. However, the extent to which hormonal mechanisms evolve alongside behavioral mechanisms and are responsible for individual variation in behavior remains under debate [7,34].

The endocrine system most studied with respect to regulatory mechanisms of personality is the hypothalamic–pituitary–adrenal (HPA) axis, which produces glucocorticoids as its principal end product. In birds, the main glucocorticoid is corticosterone

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(CORT), which serves primarily metabolic functions at baseline levels but becomes elevated shortly after the perception of a stressor, and continues to increase until a process of negative feedback permits baseline levels to be re-achieved (i.e., the endocrine stress response; see [40]). The glucocorticoid stress response is widespread, perhaps universal amongst vertebrates [17,62], and is an adaptation that enables organisms to regain homeostasis following challenges encountered in normal life.

Hormones like CORT can affect multiple regulatory and behavioral processes simultaneously. For example, elevated levels of CORT stimulate foraging and promote locomotor activity when food reserves are low, but can also suppress reproduction [40,45]. Because a single hormone, such as CORT, can generate co-variation at multiple physiological levels (i.e., hormonal pleiotropy), and because the HPA axis is known to influence behavior, the pleiotropic effects of CORT might also contribute to behavioral co-variation [2,61]. In fact, several of the behaviors that typify personality variation (e.g., shyness in non-human animals, neuroticism in humans; [21]) are thought to be related, either directly or indirectly, to differences in how animals cope hormonally with stress [27].

There is often considerable inter-individual variation in baseline and peak concentrations of CORT [10,59]. We have limited knowledge about how this potential variation relates to animal personality, particularly in non-domesticated species, but some key studies have provided testable predictions (reviewed in [9]). Specifically, shyer individuals (i.e., reactive copers) are predicted to have higher HPA axis reactivity, lower sympathetic adrenomedullary reactivity, higher cardiac parasympathetic activity, a stronger innate and adaptive immune response, and a higher susceptibility to stress-related illness compared to bolder (proactive) individuals [4,27,29,31,46,56], including in humans (reviewed in: [17]).

Artificial selection experiments provide a powerful method for identifying co-selected traits. In what has become a model system in this field, artificial selection on temperament in silver foxes demonstrated correlated selection on morphological and physiological traits, including stress physiology [24]. Likewise, artificial selection on stress physiology in birds has shown correlated changes in coping behavior. For example, Japanese quail (*Coturnix coturnix japonica*) artificially selected for low and high CORT responses [12] show variation in open field behavior, comparable to the novel environment test used in the present study. Birds from the line selected for low CORT responses showed less freezing behavior and began exploring sooner than did birds selected for high CORT responses [25].

Here, we examine the relationship between the stress response and personality in a wild vertebrate model system, the great tit, *Parus major*. In great tits, exploration behavior (in a novel environment) and boldness (toward a novel object) are phenotypically correlated, repeatable within individuals, and artificial selection experiments have demonstrated a heritable component [16]. Further, this combination of behaviors ('fast-bold'/'slow-shy') correlates phenotypically and genetically with other behavioral tendencies, such as risk-taking [52], aggression, and dominance [58]. Studies that used feces to determine hormone concentrations showed that great tits selected for a combination of slow exploration and shyness (slow explorers, 'SE') had elevated fecal CORT concentrations when challenged compared to the line of fast explorers ('FE') [5]. One additional advantage of this model system is the lack of sex differences in both exploratory behavior [6] and CORT concentrations [50], thus making inferences about correlated selection sex-independent.

Here we test the hypothesis that HPA axis reactivity underlies variation in personality by measuring plasma corticosterone responses in great tits of the two lines. In addition to testing this

hypothesis in a non-domesticated species, our approach departs from previous studies by selecting on composite behavioral traits rather than single physiological or behavioral ones. We used two complementary experiments: first, we measured CORT responses to capture and restraint in the lines of birds bi-directionally selected for personality. We predicted that the SE line would have elevated responses to stress compared to the FE line. Second, we measured corticosterone responses in a group of non-selected birds of wild origin to examine the continuous relationship between individual behavioral characteristics and corticosterone responses.

## 2. Materials and methods

### 2.1. Subjects, behavioral testing and hormone sampling

#### 2.1.1. Experiment 1: Selection line birds

In October 2007 we sampled 16 great tits (5 months old, originating from 14 broods) descended from the fourth generation of previously established lines bi-directionally selected for fast exploration and boldness (FE;  $N = 9$ : 2 females, 7 males) and slow exploration and shyness (SE;  $N = 7$ : 5 females, 2 males). The parental generation was chosen from 81 hand-raised birds (11 broods) originating from two wild populations in the Netherlands (for more details see [16]). At each generation, nestlings were combined into mixed broods and cross-fostered to adults in the wild, and then hand-raised following nutritional independence. Mating pairs (FE with FE, SE with SE) were assigned according to a composite personality score. The selection criterion used was a combined score resulting from two classes of behavioral tests: (1) a novel environment test—analogue to an open field test [57]; and (2) two tests evaluating each bird's response to novel objects. Each bird was tested individually shortly after nutritional independence (ca. 30–40 days posthatching) at the Netherlands Institute for Ecology. For the novel environment test, birds were allowed to freely enter and explore a room (2.0 × 4.0 and 2.5 m high) by exiting through an opened sliding door separating their home cage from the novel room. We monitored and recorded all movement during each trial. A trial was discontinued if a subject reached all five trees in less than 10 min, but all trials were conducted for a minimum of 2 min independent of a bird's exploratory speed. We converted the latency to perch on the fourth of five artificial trees to a linear scale from 0 to 10 and used this as the measure of exploratory tendency (hereafter, '4th tree score'; see [16,57]). A score of 10 indicates a bird that reached the 4th tree within 1 min ('fast') and a score of 0 indicating a bird that failed to reach the 4th tree within 10 min ('slow').

For each bird two novel object tests were conducted, one with a penlight battery as the object and one with a bendable Pink Panther figurine (8 cm). The object was placed on one of the outer perches of the subject's home cage and the latency to approach this perch and the minimum distance to the novel object were measured during a 120 s trial. These two measures were combined into a single novel object score along a linear scale (0–5)—this score is used as a measure of shyness (with a score of 0 representing extremely shy birds). Together, scores from these three behavioral tests (1 novel environment, and 2 novel objects) were summed and this composite score is referred to as early exploratory behavior—the score used during each round of selection (for more details on the behavioral tests and the selection procedure, see [16,52,53,57]). After behavioral testing all birds were transferred to open outdoor aviaries (2 × 4 and 2.5 m high) where they were housed in single sex groups for the rest of the year.

In autumn 2007 birds were distributed singly in cages and allowed to acclimatize undisturbed for 7 days prior to hormone

sampling. Before and during sampling birds were maintained individually in their home cages (0.9 × 0.4 and 0.5 m high), which consisted of solid walls except for a wire-mesh front, three perches, and food and water dishes. The natural light–dark cycle and ambient temperatures were maintained because rooms were equipped with large open windows that allowed for natural lighting and air-flow. Birds were provided with ad libitum water, seed mixture and calcium, and this was supplemented daily with mealworms and a mixture of sour milk, ground beef heart, commercial egg product and multivitamins.

We collected blood samples from 6 SE and 6 FE individuals each day on October 29th, 30th and 31st between 1100 and 1230. All birds had completed molt. To minimize unintended stress birds were distributed across six rooms and left undisturbed for at least 2 h prior to sampling. We used a standardized capture and restraint protocol to examine baseline and stress-induced CORT concentrations. On the day of sampling we collected approximately 75 µL of blood at each of three time points by puncturing the alar vein and the time from entering the room until finishing each blood sample was recorded. The first blood sample for baseline CORT ('CORT0') was collected in less than 3 min following entry into each room (mean ± 1 SEM (s); all: 97.3 ± 10.0; fast: 91.7 ± 13.9; slow: 104.6 ± 15.1). CORT becomes elevated after approximately 3 min in many taxa [41]. All samples exceeding this duration were excluded from analysis. Thus our final sample size included birds sampled on 29 October (5 FE, 2 SE), 30 October (4 FE, 4 SE) and 31 October (1 SE). After the baseline bleed, birds were held in small cloth bags until they were quickly rebled at 15 min ('CORT15') and 30 min post-disturbance ('CORT30'). Blood samples were kept on ice for less than 1.5 h before being centrifuged (5000 rpm for 10 min; ca. 1400 g). The plasma fraction was then stored at –80 °C until transfer on dry ice to the Max Planck Institute for Ornithology (Radolfzell, Germany) for hormone measurement.

### 2.1.2. Experiment 2: Non-selected birds

In November 2009 and October 2010 we sampled blood for CORT from 68 great tits (19 months old;  $N = 28$  in 2009: 11 females, 17 males;  $N = 40$  in 2010: 23 females, 17 males). These birds originated from a long-term study population at the Hoge Veluwe National Park (the Netherlands) and were taken into captivity as nestlings in 2008 and 2009. All nestlings were sampled for blood at 3 days old for molecular sexing [22], and to identify extra-pair offspring prior to brood selection [44]. The 2009 sample originated from 11 broods and the 2010 sample from 13 broods. On day 10 post-hatching, chicks were transferred to the Netherlands Institute for Ecology (Heteren, the Netherlands) for hand-rearing [16] as in Experiment 1.

We tested all fledglings for early exploratory behavior (ca. 30–40 days post-hatching) in the novel environment test as described in Experiment 1 (i.e. 4th tree score). Afterwards the birds were transferred to open outdoor aviaries (2 × 4 and 2.5 m high) where they were housed in single sex groups for the rest of the year. On 1 December each year, breeding pairs were formed randomly with respect to exploratory scores, but avoiding sib-matings, and then housed in climate-controlled aviaries for the next year as breeding pairs. Photoperiod and diet were the same as in Experiment 1. Our final sample also included a few individuals ( $N = 2$  in 2008,  $N = 7$  in 2009) generated from F1 and F2 crossings from the selection line birds and, as with the birds in Experiment 1, were cross-fostered and raised by wild parents until day 10 post-hatching. We included these birds because F1 and F2 crosses are expected to have a mean exploratory score in-between the two selection lines.

We collected blood samples between 0930 and 1330 on 2 and 3 November (2009) and 12 and 15 October (2010) following the completion of molt. Birds were left undisturbed in their home aviaries (as breeding pairs) for at least 2 h prior to sampling and only

1 bird from the pair was sampled on a given day. We used the same capture-restraint protocol from Experiment 1 with the following exceptions: (a) birds were captured from their home aviaries using a net rather than hand-caught in cages; (b) birds were bled at only two time points, baseline (CORT0) and 30 minutes post-capture (CORT30); (c) CORT0 samples were collected by puncture of the alar vein but CORT30 samples were collected from the jugular. Baseline bleeds not completed in less than 3 min after aviary entry are not included here (mean ± 1 SEM (s); 138.4 ± 3.1). As in Experiment 1, blood samples were kept on ice briefly, centrifuged to separate the plasma which was stored at –80 °C and transferred on dry ice to the Max Planck Institute for Ornithology (Radolfzell, Germany) for hormone measurement.

### 2.2. Enzyme immunoassay for CORT

Plasma concentrations of CORT were estimated using commercially available enzyme immunoassay (EIA) kits (Enzo Life Sciences, Cat. No. ADI 900-097; Donkey anti-Sheep IgG). Details of the EIA protocol (parallelism, precision and quantitative recovery) can be found in [36]. Concentrations were determined following a diethyl-ether extraction of a 5–10 µL sample volume. After drying extracts under a stream of N<sub>2</sub> gas, samples were diluted using Tris-buffered saline (provided by kit) and samples were re-suspended overnight at –20 °C at a 1:30 dilution. Additionally, samples of stripped chicken plasma spiked with a known quantity of purified CORT (20 ng mL<sup>-1</sup>) were extracted alongside the unknown samples for the estimation of intra- and inter-assay variation. The following day, 100 µL aliquots of each sample were added assigned to individual wells across assays with each plate containing hormone standards and a separate standard curve (range: 32–20,000 pg mL<sup>-1</sup>). Plates were then placed on an orbital shaker for 2 h to bind the antibody and CORT conjugate. We then washed the wells four times using 200 µL of wash solution and afterwards added 200 µL of *p*-nitrophenyl phosphate to each well. The plates were then developed in darkness for 1 h and 50 µL of stop solution was added to each well immediately before being read on a VERSA<sub>max</sub> microplate reader at 405 nm. We ran samples in duplicate and accepted the average of both readings as the final value for each sample. This commercial assay has a detection limit of 27 pg mL<sup>-1</sup>. The average extraction recovery in our laboratory was estimated previously to be ca. 90% as determined from individual samples spiked with a small amount of radioactively labeled CORT [36]. We therefore corrected each sample for this loss. For Experiment 1 (2 plates), the intra- and inter-assay coefficients of variation (CV) were 14.69% and 5.65%, respectively. For Experiment 2 (8 plates), the intra- and inter-assay CVs were 5.21% and 7.00%, respectively. The cross-reactivity of the antiserum is 100% for corticosterone, 28.6% for deoxycorticosterone, and 1.7% for progesterone.

### 2.3. Statistical analyses

All data in this study were analyzed using SPSS (version 16.0, SPSS Inc., Chicago, IL, USA). Following square root transformation of CORT values, the normality of the residuals was tested by visual inspection of Q–Q plots and Kolmogorov–Smirnov tests and homogeneity of variances were examined with Levene's test. The assumptions of all test statistics were met unless indicated otherwise. All tests were two-tailed and significance was determined at the  $\alpha = 0.05$  level. Data are presented as mean ± 1 SEM.

#### 2.3.1. Experiment 1: Selection line birds

Differences in tarsus length (as a measure of body size) were examined using a univariate analysis of variance and the influence of tarsus length on CORT concentrations was tested with Pearson's



correlations. Differences in early exploratory behavior were tested using non-parametric Mann-Whitney U-tests. We used a repeated measures general linear model to examine the influence of selection line on CORT across the three time points. To avoid overparameterization with these limited sample sizes we did not examine sex differences; however, previous work with these selection lines has shown that males and females do not differ in exploratory behavior [6] or CORT concentrations [50] (see also Experiment 2 in Results).

### 2.3.2. Experiment 2: Non-selected birds

We first tested for differences in 4th tree scores between the 2009 and 2010 datasets using a Mann-Whitney U-test. We used univariate GLMs to test for year and sex effects on CORT0 and CORT30. Finally, we tested for a relationship between 4th tree scores and CORT0 and CORT30 using the non-parametric Spearman's rank correlations.

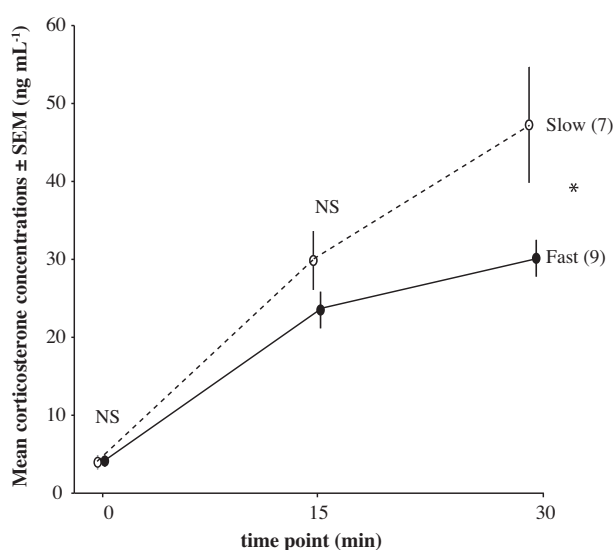
### 2.4. Ethical note

This study was carried out in accordance with the animal ethical committee of the Royal Dutch Academy of Sciences (DEKNAW) under protocols CTE.0705 and CTE.0707 (Experiment 1) and CTE.0809 and CTE.0907 (Experiment 2).

## 3. Results

### 3.1. Experiment 1: Selection line birds

The selection lines differed in early exploratory behavior (mean composite score  $\pm$  1 SEM: slow =  $10.13 \pm 1.21$ ; fast =  $12.55 \pm 1.02$ ; Mann-Whitney U = 11.5,  $p = 0.031$ ) as has been shown previously for these selection lines [16]. Plasma concentrations of CORT increased significantly across the three blood sampling time points ( $F_{2,13} = 163.7$ ,  $p < 0.001$ ; Fig. 1). The main effect of selection line on CORT concentrations was not significant ( $F_{1,14} = 3.64$ ,  $p = 0.077$ ) but the interaction between selection line and sampling time point was significant ( $F_{2,13} = 5.25$ ,  $p = 0.012$ ). Planned comparisons (Sidak corrected) between selection lines yielded signifi-



**Fig. 1.** Experiment 1. Baseline and stress-induced corticosterone concentrations for fast-bold (solid,  $N = 9$ ) and slow-shy (dashed,  $N = 7$ ) selection line birds evaluated using a within-subject design (mean  $\pm$  1 SEM). Concentrations increased significantly at each time point, and differed between selection lines at 30 min (NS = non-significant; \* $p < 0.05$ ).

cantly higher CORT levels in SE at the CORT30 time point ( $p = 0.017$ ). Similarly, the SE line had higher CORT concentrations at 15 min but this difference was not statistically significant ( $p = 0.161$ ). No differences were observed for CORT0 ( $p = 0.824$ ).

Tarsus length did not differ between individuals from FE (mean  $\pm$  SD;  $20.11 \pm 0.46$  mm,  $N = 9$ ) and SE ( $19.74 \pm 0.53$  mm,  $N = 7$ ) lines ( $F_{1,12} = 0.089$ ,  $p = 0.771$ ). We observed a sex difference in tarsus length ( $F_{1,12} = 5.124$ ,  $p = 0.043$ ) but there was no interaction between sex and line ( $F_{1,12} = 0.328$ ,  $p = 0.577$ ). Mass measurements at the time of blood sampling were not available, making a comparison of body condition between lines not possible; however, mass, body condition and tarsus have been shown previously not to differ between the selection line birds [56]. Further, CORT concentrations were not related to tarsus length at any of the three sampling time points (Pearson correlations, all  $R^2 < 0.04$ , and all  $p > 0.5$ ).

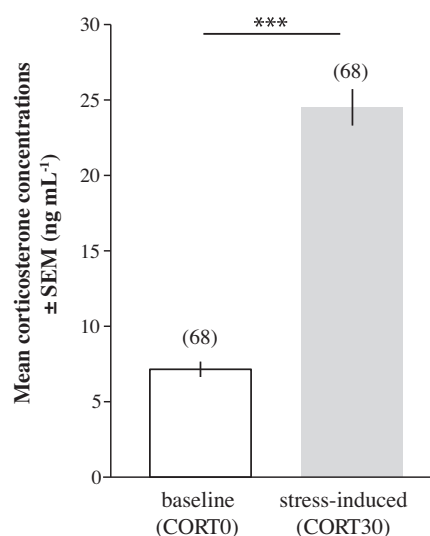
### 3.2. Experiment 2: Non-selected birds

Concentrations of CORT significantly increased between the baseline and stress-induced sample (paired  $t$ -test:  $t_{67} = 18.16$ ,  $p < 0.001$ ; Fig. 2). There was no correlation between 4th tree scores and CORT0 or CORT30 (Spearman's rank correlations: CORT0:  $\rho = -0.05$ ,  $p = 0.684$ ; Fig. 3a; CORT30:  $\rho = -0.03$ ,  $p = 0.781$ ; Fig. 3b).

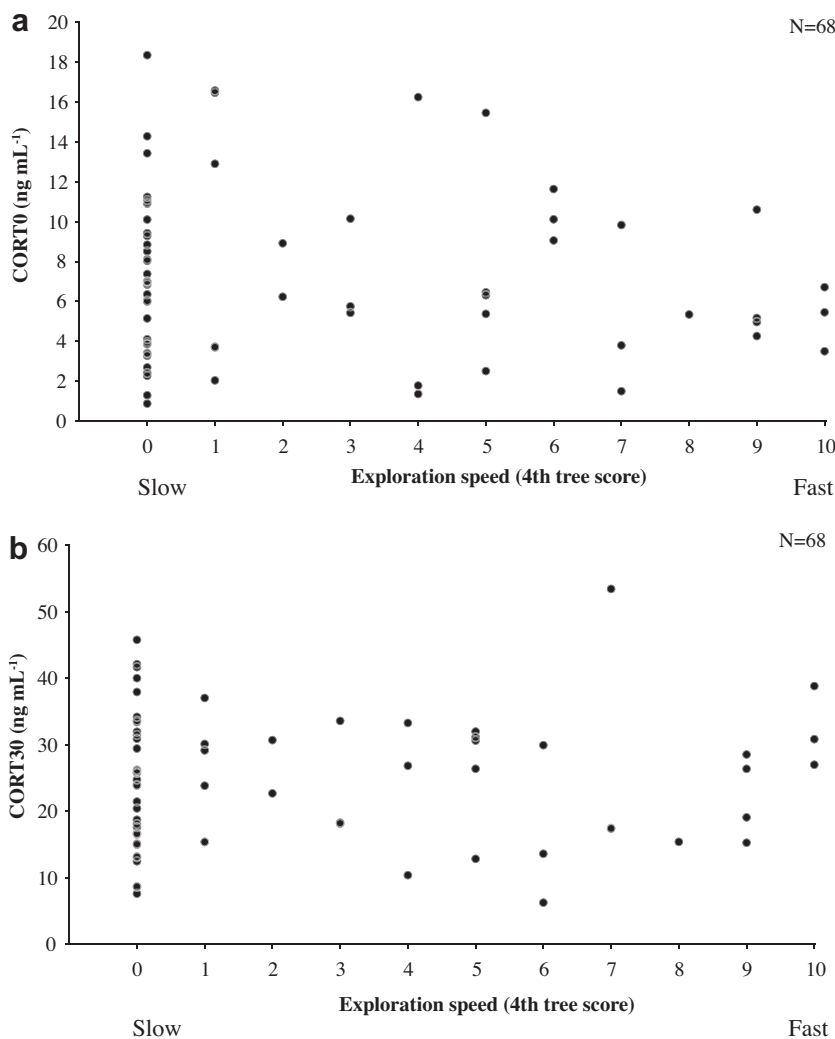
There were no differences in 4th tree scores between birds in 2009 and 2010 (Mann-Whitney U = 526.5,  $p = 0.485$ ). Likewise, there were no differences between years in CORT0 ( $F_{1,63} = 1.079$ ,  $p = 0.303$ ) or CORT30 ( $F_{1,63} = 0.03$ ,  $p = 0.866$ ). The sexes did not differ in CORT at either time point (CORT0:  $F_{1,63} = 0.018$ ,  $p = 0.893$ ; CORT30:  $F_{1,63} = 0.458$ ,  $p = 0.875$ ), nor was there a significant interaction between sex and year in the model (CORT0:  $F_{1,63} = 0.995$ ,  $p = 0.322$ ; CORT30:  $F_{1,63} = 0.025$ ,  $p = 0.875$ ).

## 4. Discussion

We used a model organism for the study of animal personality to test the prediction that slower-shyer individuals have greater HPA reactivity compared to faster-bolder birds. Because variation in both personality [16] and stress physiology [18,35] can have a heritable basis in birds, testing for correlated selection on these two types of complex traits as we have done here is warranted.



**Fig. 2.** Experiment 2. Corticosterone concentrations increased significantly between baseline and stress-induced samples in non-selected birds (mean  $\pm$  1 SEM;  $N = 68$ ; \*\*\* $p < 0.001$ ).



**Fig. 3.** Experiment 2. There was no continuous relationship between 4th tree scores and (a) baseline corticosterone (CORT0) or (b) stress-induced corticosterone (CORT30) concentrations in non-selected birds ( $N = 68$ ).

We found that great tits artificially selected for a combination of slow exploratory behavior and shyness toward novel objects reach higher circulating CORT concentrations following exposure to a standardized stressor compared to individuals from the fast-bold selection line. The direction of the effect on stress-induced CORT levels corroborates patterns found in other vertebrate species [7,27], as well as two previous studies that examined fecal CORT from great tit selection lines. Carere et al. [5] found that adults of the SE line had elevated fecal CORT concentrations following a simulated territorial intrusion (a potent social stressor in this species; [51]), while no differences were observed during the non-stressor treatment. A study by Stöwe et al. [50] showed that great tit nestlings of a SE line had generally elevated fecal CORT concentrations. Fecal CORT measurements, however, represent a combination of stressed and non-stressed contributions [33,37]. Therefore by showing that baseline levels of plasma CORT do not differ, while stress-induced levels do, we provide some support for the idea that it is reactivity in the HPA system, and not basal regulation, that correlates with personality.

There was no relationship between a measure of exploratory behavior (4th tree score) and baseline corticosterone or the corticosterone response to a stressor in non-selected birds. In other species, birds with relatively proactive or reactive personalities show relatively less or more fearfulness [9], and fearfulness is one behav-

ioral characteristic associated with corticosterone. For example, white chickens are considered to be more fearful than brown chickens, and white Leghorn chickens had higher corticosterone responses and showed greater fearfulness than brown Hyline hens [20]. However, correlations between behavioral measures of fearfulness or exploratory behavior and corticosterone have been found in some avian studies [25,26,31] but not others [19,32]. Fearfulness and personality cannot be readily measured using a single test [9], and the absence of a correlation between a single measure of exploratory behavior and corticosterone responses in the great tits does not preclude the existence of a general relationship between other aspects of personality and corticosterone in this species. Selection line birds generally had narrower distributions for exploratory scores and CORT concentrations compared to the non-selected birds, potentially reflecting the disrupting (bi-directional) process of selection on both phenotypic characters (see [Supplementary materials S1–S3](#)).

Together the studies conducted in great tits agree generally with a broader picture that is emerging for vertebrates: shyer phenotypes exhibit a greater reactivity of the HPA axis as demonstrated by a higher corticosterone concentration in response to strong stressors [27,31,62]. In birds, a capture-restraint protocol physically constrains subjects into highly stressful conditions without permitting much freedom to cope by employing a

particular behavioral strategy. Differences in the stress reactivity between individuals might be diminished, or alternatively enhanced, if allowed to cope behaviorally [17]. Future studies will thus need to extend this line of research to include stressors—particularly naturalistic ones—that allow subjects to cope in an active or passive way. Indeed, it is precisely these coping strategies that are predicted to vary amongst personality types [28], and therefore such an experimental design would be complementary to a restraint style stressor. Here, monitoring the behavioral response in conjunction with the endocrine response will be essential for teasing apart these two possible contributions to differences across personalities. It is important to note that the present selection line study and similar ones before it provide correlational evidence for a link between personality and HPA reactivity. This finding does not necessitate, however, that variation in plasma CORT is responsible for the behavioral variation observed during the exploration trials. Given that differences in plasma CORT were not observed until the 30 min time point, it is quite likely that the behavioral differences are the product of a more rapidly acting physiological response. Future studies, therefore, will need to determine if upstream regulatory centers in the brain respond to selection and are directly linked to personality.

In conclusion, we have shown that phenotypic selection on a combination of two personality traits (exploration and boldness) is associated with differences in the physiological stress response, a result we think reflects the idea that selection on personality structure—and not simply a single behavioral response—is key. Van Oers et al. [56] examined a similar question using selection line great tits and found a correlation between personality and plasma testosterone levels. Together, this might suggest that a suite of hormonal traits is associated with a suite of behavioral traits. Future studies examining the link between animal personality and stress physiology should therefore also consider exploring other components of the HPA cascade, and the targets of CORT. For example, glucocorticoid and mineralocorticoid receptor expression in the hypothalamus in birds can be sensitive to differences in HPA axis reactivity [13]. Slow birds might be more sensitive to the perception of stressors, secreting more corticosterone releasing factor in response to a standardized stressor, and thereby exhibiting an earlier onset of the corticosterone stress response; an alternative, which is not mutually exclusive, is that slower phenotypes have a dampened negative feedback control of CORT causing them to achieve higher stress-induced levels. At a different regulatory level, the serotonergic system has been at the center of coping biology, and while much of the attention has been focused on laboratory mammals, personality researchers working with wild taxa would benefit from examining both HPA and serotonin systems simultaneously [28]. These are exciting avenues for future work, especially considering recent evidence demonstrating that both personality characteristics (reviewed in [14]) and how animals respond physiologically and behaviorally to natural stressors is important to their health, survival and reproductive success [3,8,23,43].

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygcen.2011.12.012.

### References

- [1] A.M. Bell, S.J. Hankison, K.L. Laskowski, The repeatability of behaviour: a meta-analysis, *Anim. Behav.* 77 (2009) 771–783.
- [2] P.A. Biro, J.A. Stamps, Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.* 23 (2008) 361–369.
- [3] C.W. Breuner, S.H. Patterson, T.P. Hahn, In search of relationships between the acute adrenocortical response and fitness, *Gen. Comp. Endocrinol.* 157 (2008) 288–295.
- [4] C. Carere, K. Van Oers, Shy and bold great tits (*Parus major*): body temperature and breath rate in response to handling stress, *Physiol. Behav.* 82 (2004) 905–912.
- [5] C. Carere, T.G.G. Groothuis, E. Möstl, S. Daan, J.M. Koolhaas, Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress, *Horm. Behav.* 43 (2003) 540–548.
- [6] C. Carere, P.J. Drent, L. Privitera, J.M. Koolhaas, T.G.G. Groothuis, Personalities in great tits *Parus major*: stability and consistency, *Anim. Behav.* 70 (2005) 795–805.
- [7] C. Carere, D. Caramaschi, T.W. Fawcett, Covariation between personalities and individual differences in coping with stress: converging evidence and hypotheses, *Curr. Zool.* 56 (2010) 728–740.
- [8] S.A. Cavigelli, Animal personality and health, *Behaviour* 142 (2005) 1129–1250.
- [9] J.F. Cockrem, Stress, corticosterone responses and avian personalities, *J. Ornithol.* 148 (2007) S169–S178.
- [10] J.F. Cockrem, B. Silverin, Variation within and between birds in corticosterone responses of great tits (*Parus major*), *Gen. Comp. Endocrinol.* 125 (2002) 197–206.
- [11] J.F. Cockrem, D.P. Barrett, E.J. Candy, M.A. Potter, Corticosterone responses in birds: Individual variation and repeatability in Adelie penguins (*Pygoscelis adeliae*) and other species, and the use of power analysis to determine sample sizes, *Gen. Comp. Endocrinol.* 163 (2009) 158–168.
- [12] J.F. Cockrem, E.J. Candy, S.A. Castille, D.G. Satterlee, Plasma corticosterone responses to handling in Japanese quail selected for low or high plasma corticosterone responses to brief restraint, *Brit. Poult. Sci.* 51 (2010) 453–459.
- [13] M.J. Dickens, L.M. Romero, N.E. Cyr, S.L. Meddle, Chronic stress alters glucocorticoid receptor and mineralocorticoid receptor mRNA expression in the European starling (*Sturnus vulgaris*) brain, *J. Neuroendocrinol.* 21 (2009) 832–840.
- [14] N.J. Dingemanse, D. Réale, Natural selection and animal personality, *Behaviour* 142 (2005) 1165–1190.
- [15] N.J. Dingemanse, C. Both, P.J. Drent, J.M. Tinbergen, Fitness consequences of avian personalities in a fluctuating environment, *Proc. R. Soc. Lond. Ser. B* 271 (2004) 847–852.
- [16] P.J. Drent, K. Van Oers, A.J. Van Noordwijk, Realised heritability of personalities in the great tit (*Parus major*), *Proc. R. Soc. Lond. Ser. B* 270 (2003) 45–51.
- [17] B.J. Ellis, J.J. Jackson, W.T. Boyce, The stress response systems: universality and adaptive individual differences, *Develop. Rev.* 26 (2006) 175–212.
- [18] M.R. Evans, M.L. Roberts, K.L. Buchanan, A.R. Goldsmith, Heritability of corticosterone response and changes in life history traits during selection in the zebra finch, *J. Evol. Biol.* 19 (2005) 343–352.
- [19] J.M. Faure, D. Val-Laillet, G. Guy, M.D. Bernadet, D. Guemene, Fear and stress reactions in two species of duck and their hybrid, *Horm. Behav.* 43 (2003) 568–572.
- [20] F. Fraisse, J.F. Cockrem, Corticosterone and fear behaviour in white and brown caged laying hens, *Brit. Poult. Sci.* 47 (2006) 110–119.
- [21] S.D. Gosling, From mice to men: What can we learn about personality from animal research? *Psychol. Bull.* 127 (2001) 45–86.
- [22] R. Griffiths, M.C. Double, K. Orr, J.G. Dawson, A DNA test to sex most birds, *Mol. Ecol.* 7 (1998) 1071–1075.
- [23] M. Hau, R.E. Ricklefs, M. Wikelski, K.A. Lee, J.D. Brawn, Corticosterone, testosterone and life history strategies of birds, *Proc. R. Soc. Lond. Ser. B* 277 (2010) 3203–3212.
- [24] M. Harri, J. Mononen, L. Ahola, I. Plyusnina, T. Rekilä, Behavioural and physiological differences between silver foxes selected and not selected for domestic behaviour, *Anim. Welfare* 12 (2003) 305–314.
- [25] R.B. Jones, D.G. Satterlee, F.H. Ryder, Fear and distress in Japanese quail chicks of two lines genetically selected for low and high adrenocortical response to immobilization stress, *Horm. Behav.* 26 (1992) 385–393.
- [26] R.B. Jones, D.G. Satterlee, R.H. Ryder, Fear of humans in Japanese quail selected for low or high adrenocortical response, *Physiol. Behav.* 56 (1994) 379–383.
- [27] J.M. Koolhaas, S.M. Korte, S.F. De Boer, B.J. Van Der Vegt, C.G. Van Reenen, H. Hopster, I.C. De Jong, M.A.W. Ruis, H.J. Blokhuis, Coping styles in animals: current status in behavior and stress-physiology, *Neurosci. Biobehav. Rev.* 23 (1999) 925–935.
- [28] J.M. Koolhaas, S.F. de Boer, B. Buwalda, K. Van Reenen, Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms, *Brain Behav. Evol.* 70 (2007) 218–226.

- [29] S.M. Korte, G. Beuving, W. Ruesink, H.J. Blokhuis, Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens, *Physiol. Behav.* 62 (1997) 437–441.
- [30] S.M. Korte, J.M. Koolhaas, J.C. Wingfield, B.S. McEwen, The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease, *Neurosci. Biobehav. Rev.* 29 (2005) 3–38.
- [31] T.L.F. Martins, M.L. Roberts, I. Giblin, R. Huxham, M.R. Evans, Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches, *Horm. Behav.* 52 (2007) 445–453.
- [32] S. Mignon-Grasteau, F. Minvielle, Relation between tonic immobility and production estimated by factorial correspondence analysis in Japanese quail, *Poult. Sci.* 82 (2003) 1839–1844.
- [33] J.J. Millspaugh, B.E. Washburn, Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation, *Gen. Comp. Endocrinol.* 138 (2004) 189–199.
- [34] A. Mutzel, B. Kempenaers, S. Laucht, N.J. Dingemanse, J. Dale, Circulating testosterone levels do not affect exploration in house sparrows: observational and experimental tests, *Anim. Behav.* 81 (2011) 731–739.
- [35] F.M. Odeh, G.G. Cadd, D.G. Satterlee, Genetic characterization of stress responsiveness in Japanese quail. 2. Analyses of maternal effects, additive sex linkage effects, heterosis, and heritability by diallel crosses, *Poult. Sci.* 82 (2003) 31–35.
- [36] J.Q. Ouyang, M. Hau, F. Bonier, Within seasons and among years: when are corticosterone levels repeatable? *Horm. Behav.* (2011) doi:10.1016/j.yhbeh.2011.08.004.
- [37] R. Palme, E. Möstl, Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood, *Mamm. Biol.* 62 (Suppl. II) (1997) 192–197.
- [38] D. Réale, S.M. Reader, D. Sol, P.T. McDougall, N.J. Dingemanse, Integrating animal temperament within ecology and evolution, *Biol. Rev.* 82 (2007) 291–318.
- [39] M.A. Rensel, S.J. Schoech, Repeatability of baseline and stress-induced corticosterone levels across early life stages in the Florida scrub-jay (*Aphelocoma coerulescens*), *Horm. Behav.* 59 (2011) 497–502.
- [40] L.M. Romero, Physiological stress in ecology: lessons from biomedical research, *Trends Ecol. Evol.* 19 (2004) 249–255.
- [41] L.M. Romero, J.M. Reed, Collecting baseline corticosterone samples in the field: is under three minutes good enough? *Comp. Biochem. Physiol. Part A: Mol. Integ. Physiol.* 140 (2005) 73–79.
- [42] L.M. Romero, J.M. Reed, Repeatability of baseline corticosterone concentrations, *Gen. Comp. Endocrinol.* 156 (2008) 27–33.
- [43] L.M. Romero, M. Wikelski, Stress physiology as a predictor of survival in Galapagos marine iguanas, *Proc. R. Soc. Lond. Ser. B* 277 (2010) 3157–3162.
- [44] V. Saladin, D. Bonfils, T. Binz, H. Richner, Isolation and characterization of 16 microsatellite loci in the Great Tit *Parus major*, *Mol. Ecol. Notes* 3 (2003) 520–522.
- [45] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, *Endocrin. Rev.* 21 (2000) 55–89.
- [46] D.G. Satterlee, W.A. Johnson, Selection of Japanese quail for contrasting blood corticosterone response to immobilization, *Poult. Sci.* 67 (1988) 25–32.
- [47] W. Schuett, T. Tregenza, S.R.X. Dall, Sexual selection and animal personality, *Biol. Rev.* 85 (2010) 217–246.
- [48] A. Sih, A. Bell, J.C. Johnson, Behavioral syndromes: an ecological and evolutionary overview, *Trends Ecol. Evol.* 19 (2004) 372–378.
- [49] B.R. Smith, D.T. Blumstein, Fitness consequences of personality: a meta-analysis, *Behav. Ecol.* 19 (2008) 448–455.
- [50] M. Stöwe, B. Rosivall, P.J. Drent, E. Möstl, Selection for fast and slow exploration affects baseline and stress-induced corticosterone excretion in Great tit nestlings *Parus major*, *Horm. Behav.* 58 (2010) 864–871.
- [51] E. Van Duysel, R. Pinxten, V.M. Darras, L. Arckens, M. Eens, Opposite changes in plasma testosterone and corticosterone levels following simulated territorial challenge in male Great tits, *Behaviour* 141 (2004) 451–467.
- [52] K. Van Oers, P.J. Drent, P. de Goede, A.J. Van Noordwijk, Realized heritability and repeatability of risk-taking behaviour in relation to avian personalities, *Proc. R. Soc. Lond. Ser. B* 271 (2004) 65–73.
- [53] K. Van Oers, G. De Jong, P.J. Drent, A.J. Van Noordwijk, A genetic analysis of avian personality traits: correlated, response to artificial selection, *Behav. Genet.* 34 (2004) 611–619.
- [54] K. Van Oers, G. de Jong, A.J. van Noordwijk, B. Kempenaers, P.J. Drent, Contribution of genetics to the study of animal personalities: a review of case studies, *Behaviour* 142 (2005) 1191–1212.
- [55] K. Van Oers, P.J. Drent, N.J. Dingemanse, B. Kempenaers, Personality is associated with extrapair paternity in great tits *Parus major*, *Anim. Behav.* 76 (2008) 555–563.
- [56] K. Van Oers, K.L. Buchanan, T.E. Thomas, P.J. Drent, Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality, *Anim. Behav.* 81 (2011) 1055–1061.
- [57] M.E.M. Verbeek, P.J. Drent, P.R. Wiepkema, Consistent individual differences in early exploratory behaviour of male great tits, *Anim. Behav.* 48 (1994) 1113–1121.
- [58] M.E.M. Verbeek, A. Boon, P.J. Drent, Exploration, aggressive behaviour and dominance in pair-wise confrontations of juvenile male great tits, *Behaviour* 133 (1996) 945–963.
- [59] H. Wada, K.G. Salvante, C. Stables, E. Wagner, T.D. Williams, C.W. Breuner, Adrenocortical responses in zebra finches (*Taeniopygia guttata*): individual variation, repeatability, and relationship to phenotypic quality, *Horm. Behav.* 53 (2008) 472–480.
- [60] G.M. While, C. Isaksson, J. McEvoy, D.L. Sinn, J. Komdeur, E. Wapstra, T.G.G. Groothuis, Repeatability of intra-individual variation in plasma testosterone concentration and its sex-specific link to aggression in a social lizard, *Horm. Behav.* 58 (2010) 208–213.
- [61] T.D. Williams, Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean', *Phil. Trans. R. Soc.* 363 (2008) 1687–1698.
- [62] J.C. Wingfield, Control of behavioural strategies for capricious environments, *Anim. Behav.* 66 (2003) 807–815.