Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches

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Abstract

The existence of consistent individual differences in behavioral strategies ("personalities" or coping styles) has been reported in several animal species. Recent work in great tits has shown that such traits are heritable and exhibit significant genetic variation. Free-living birds respond to environmental stresses by up-regulating corticosterone production. Behavior during mild stress can occur in accordance to two types of coping styles, i.e. active and passive. Using artificially selected lines of zebra finches that vary in the amount of corticosterone produced in response to a manual restraint stressor we ran three "personality" experiments. We show that birds in the different corticosterone lines differ in their exploratory and risk-taking behaviors. There was an increase in exploratory behavior as corticosterone titre increased but only in the low corticosterone line. Birds in high corticosterone line showed greater risk-taking behavior than birds in the other lines. Thus, in general, higher levels of circulating corticosterone following a mild stress result in greater exploratory behavior and greater risk taking. This study shows that lines of animals selected for endocrine hormonal responses differ in their "coping" styles or "personalities".
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Introduction

Animals are often subjected to unpredictable variation in their environments and their ability to cope with this can be an important determinant of fitness. The "coping" strategies used in response to environmental changes have been shown to differ non-randomly among individuals, with behavioral and physiological reactions often correlating (Gosling, 2001; Koolhaas et al., 1999). These consistent strategies are referred to as "personalities" or coping styles when physiological stress responses are also measured. Coping styles are often polarized into two extremes and have received different "labels" in the literature, i.e. shyness–boldness (Carere and van Oers, 2004; Wilson et al., 1993), reactive–proactive (Koolhaas et al., 1999), slow–fast (Dingemanse and de Goede, 2004), passive–active (Drent et al., 2003) and anxious–less anxious (Landgraf et al., 1999).

In a variety of species, coping strategies have been shown to be subject to selection and form part of an adaptive response to changes in the environment (Koolhaas et al., 1999; Sih et al., 2004). These responses have been described as behavioral syndromes, i.e. consistent sets of individual differences in behavior which are correlated over time and/or across contexts and are expected to have significant ecological and evolutionary significance (Dall, 2004; Sih et al., 2004). Recently, coping styles have been identified in a variety of species, including in the wild (Dingemanse et al., 2004, 2002, 2003; Dingemanse and de Goede, 2004; Drent et al., 2003) and shown to have a high heritability (Drent et al., 2003; van Oers et al., 2005).

For our study, we chose to follow Drent et al. (2003) and have used the terms active and passive for coping strategies. Active strategies are characterized by fast but superficial exploratory behavior, boldness to novelties, taking risks against predators and rivals, having a relatively high chance of winning fights and becoming dominant in groups (Dingemanse and de Goede, 2004). Passive strategies are characterized by slow but thorough exploratory behavior, being relatively sensitive to changes in the surroundings, cautious to novelties, taking fewer

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risks, having relatively low chances of winning fights and becoming the highest ranking animal in the group (Carere and van Oers, 2004; Dingemanse et al., 2004; Drent et al., 2003; Koolhaas et al., 1999). Coping styles in great tits have been shown to have fitness consequences, most notably to influence reproductive success and survival (Both et al., 2005; Dinge-manse et al., 2004a; Dingemanse and de Goede, 2004b). Great tits with passive and active coping styles seem to differ in their breath rate, pointing out the strong physiological basis to coping styles (Carere and van Oers, 2004). Interestingly, physiological variations in the response to stress have been considered in laboratory studies of coping styles for many years (Koolhaas et al., 1999). For example, hens from low pecking lines thought to reflect a passive coping style had higher basal and stress-induced plasma corticosterone levels than hens from high pecking lines, thought to be active coping (Korte et al., 1997; van Hierden et al., 2002). In general, active individuals show higher norepinephrine, but lower glucocosticosterone reactivity in response to stressful situations than passive individuals (Korte et al., 1997; Carere et al., 2003; Koolhaas et al., 1999).

The main physiological stress hormone in birds, corticoste-rone has been shown to be repeatable and heritable in several captive and wild species (Cockrem, 2006; Cockrem and Silverin, 2002; Evans et al., 2006) and has been suggested as the physiological basis for coping styles (Cockrem, 2006; Kralj-Fišer et al., 2007). Individuals differ in stress-induced corti-costerone levels (Piazza et al., 1993; Schwabl, 1995).

Corticosterone levels can be interpreted as an adaptive response to stressful events. Selection can act on patterns of hormone secretion as shown by the fact that individuals in populations that are frequently exposed to environmental stresses tend to have lower corticosterone production in response to a standard stressor than individuals in populations that are infrequently exposed to such stresses (Wingfield et al., 1995). Selection in such cases has acted to modulate the physiological response to stress in a way that is appropriate for the environment. Artificial selection experiments can be useful as they mimic the process of adaptive selection, with the advantage that the selection pressure would be the only aspect of the animal's environment to have changed. The use of such artificially selected lines can help clarify the physiological mechanisms underlying coping styles or personalities (Evans et al., 2006; Schjolden et al., 2005).

In this study, we have used artificially selected divergent lines of zebra finches, *Taenopygia guttata*, that vary in peak corticosterone titre in response to a manual restraint stressor (Evans et al., 2006). We have tested six lines of zebra finches (two lines selected for high corticosterone, two for low and two control lines) for exploratory and risk-taking behavior. The use of these lines on standard exploratory tests will help to clarify if coping styles are linked to different levels of circulating hormones. As these birds have been selected to show different levels of corticosterone, we are not asking if passive or active individuals vary in their corticosterone titre but rather if birds which differ in peak corticosterone response behave in a passive or active way in exploratory and risk-taking tests (Drent et al., 2003; van Oers et al., 2004). Similar studies have been carried out for rainbow trout (Øverli et al., 2002; Overli et al., 2006; Schjolden et al., 2005).

Materials and methods

Study species, selection procedure and husbandry

Zebra finches were selectively bred at the University of Stirling, United Kingdom, for five generations from July 1999 until December 2003. Duplicate independent lines of zebra finches were selected for high, low and random (control) levels of corticosterone production (six lines in total). Birds were selected according to peak corticosterone production following a standardized capture/restraint technique (Wingfield et al., 1994). Corticosterone was sampled at 6 weeks post-fledging when the birds were independent of their parents. They had also completed a partial molt into adult plumage by this time and so could be sexed on plumage characters. Testosterone was assayed in males at 6 months. This time point was chosen as males at this age were sexually mature and would be breeding, if given access to females. Blood samples for the two hormones were not taken at the same time in order to minimize sampled blood volume. Blood samples were taken from the brachial vein after 20 min restraint in a small cloth bag (20 × 30 cm). This was identified as the time for acute stress reaction, i.e. the time for peak levels of corticosterone in the blood (Evans et al., 2006). They were placed in individual cloth bags immediately after capture. This is the standard capture–restraint protocol used in studies of the corticosterone response (Wingfield et al., 1994). Blood sampling any one bird took approximately 30 s and 100 μl of blood was taken in heparinized glass capillaries. The blood was centrifuged at $11,000 \times g$ for 15 min and the plasma frozen at -20 °C. Plasma samples from the birds were assayed within approximately 3 months of drawing, using standard radioimmunoassay techniques. Corticosterone concentrations were measured after extraction of 20 μl aliquots of plasma in diethyl ether, by radioimmunoassay (Maddocks et al., 2001; Wingfield et al., 1992). Anti-corticosterone antisera (code B21–42 and B3–163 Esoterix Inc. Endocrinology, CA) and [1,2,6,7-3H]-corticosterone label (Amer-sham, UK) were used throughout. The interassay coefficient of variation was 15.7%, and the intra-assay coefficient of variation was 3.1%. The mean extraction efficiency was 72%. The assay was run with 50% binding at 134 pg/tube, and the detection limit (for 7.3 μl aliquots of extracted plasma) was 1.76 nmol L^{-1} (Evans et al., 2006). Corticosterone titre measurements used in this study are from the standardized capture/restraint technique.

Birds used in this study were from generation 5 and all lines were housed together in a large outside aviary giving approximately 1 m^3 per breeding pair (Jones et al., 1995). The birds were provided with *ad libitum* seeds (foreign finch mixture, Haith's Ltd., Cleethorpes, Lincolnshire, UK), Chinese millet sprays, mineralized grit, water and cuttlefish bone. The finches were provided with c10g of a 3:1 mixture of "nectarblend" (Haith's Ltd., Cleethorpes, Lincolnshire, UK) and egg biscuit food (Haiths Ltd., Cleethorpes, Lincolnshire, UK) and either lettuce or cucumber daily with a cod liver oil supplement in the seed weekly. For the birds in generation 5, there was a significant 2- to 3-fold difference in mean corticosterone between high and low lines (with realized heritability of about 20%; Evans et al., 2006). Likewise, corticosterone titre (nmol L^{-1}) differed significantly between the lines for the sub-sample of birds used in this work. Because the peak corticosterone titre is achieved between 10 and 20 min, handling it is expected that tests used in the paper would have produced a peak corticosterone response. During selection procedure, a sub-sample of birds in the six selected lines was tested for testosterone levels but no differences were found, i.e. the birds differ in peak corticosterone levels but not in testosterone levels (Evans et al., 2006). These patterns were also present in the sub-sample of birds observed in this study. For the exploratory or novelty tests, a total of 57 birds were observed, 19 birds with low mean corticosterone levels, 18 birds with control mean corticosterone levels and 20 birds with high mean corticosterone levels. The mean corticosterone titres for this subset were as follows: low = 12.21 nmol L^{-1}, SE = 4.08; control = 21.87 nmol L^{-1}, SE = 4.19; high = 30.70 nmol L^{-1}, SE = 3.981 ($F_{2,54}$ = 5.53, P = 0.008). The same birds were sampled for the risk-taking or startle test but as seven birds had died before the test only 50 birds were available (17 low, 16 control and 17 high). Mean corticosterone titre (nmol L^{-1}) differed significantly between groups for this subset as well (low = 12.52 nmol L^{-1}, SE = 4.31; control = 22.18 nmol L^{-1}, SE = 4.44; and high = 28.77 nmol L^{-1}, SE = 4.31; $F_{2,47}$ = 3.60; P = 0.035).

Exploratory or novelty tests

Novel environment

Following the study of Drent and colleagues (2003), two different tests for response to a novel environment were performed. These tests were carried out in an aviary (measuring approximately 1.5 m^3) adjacent to where the finches were housed (hereafter called the test cage). Prior to this investigation none of the zebra finches had been exposed to the novel environment tests or to the test cage. All birds used in this test were provided *ad libitum* food both before and after the test. For the first test five small white empty feeding troughs were placed in the centre of five randomly selected areas of the test cage. Zebra finches were caught individually and placed in this novel environment for 10 min. The time taken to visit four of the five empty feeders was recorded and converted to a linear scale of 0 to 10 (0:00–0:59=10, 1:00–1:59=9, etc.). A score of 10 (fast) meant that the bird reached all four feeders within 1 min and a score of 0 meant that the bird did not reach the fourth feeder within 10 min (slow). As only a few of our birds reached 4 feeders in 10 min, we also recorded the number of feeders visited within the 10-min test period.

Novel objects

The second test was of behavior in response to two different novel objects placed in the centre perch in their home cage. The first novel object was a small AA size battery and the second object was a bright green purse measuring 9 by 11 cm. The birds had not encountered any objects similar to these previously. All birds used in this test were provided *ad libitum* food both before and after the test. Following the study of Drent and colleagues (2003), test birds were caught and introduced to the first object on the first test day and to the second on the second day. Birds were caught at random during both days. For both tests, the behavior of the test bird towards the novel object was recorded from behind a wall for the first 2 min after the bird was introduced in the home cage. The reaction to both objects was recorded on a scale of 0 to 5, where 5 was to land on the object or peck it and 0 was to perch as far as possible from the object, test score was the maximum rating achieved within the first 2 min after being placed in the cage. A bird's overall score was the sum of the scores for the two objects. Thus, the maximum score a bird could achieve from this second test was 10 if it landed on both objects and the minimum was 0 if it did not land anywhere near either object in the time available.

Analyses were carried out on both scores separately (novel environment and novel objects) and on the sum of the scores achieved in both the novelty tests (Drent et al., 2003; van Oers et al., 2004), where any birds scoring 0 were extremely slow and any birds scoring 20 would have been extremely fast. Very few of our birds reached 4 empty feeders in 10 min of the novel environment test, thus in practice the maximum score recorded was 10.

During the novelty tests, focal birds were out of visual contact with their flock mates which were left in the wooden shed adjacent to the home cage.

Risk taking or startle test

Risk-taking behavior was tested as latency to return after a mild startle in a food context following the method used by van Oers and colleagues (2004). As the test performed here was designed for great tits (Drent et al., 2003; van Oers et al., 2004), a territorial species, and zebra finches are highly social birds, we decided to include a "companion bird" in the aviary for the startle test (van Oers et al., 2005). This latency test took place 8–10 weeks after the novel environment (standard) tests. All birds tested were provided with *ad libitum* food prior to the test. For this test we placed a feeding station (30×30 cm) equipped with a hinged wooden lid attached to the side of the aviary. In the middle of this station we placed a feeding tray (22×30 cm) filled with foreign finch seed mix (John E. Haith Ltd., UK). The lid was controlled from outside the aviary via a cord. While the cord was tensioned it kept the lid up and the access to the feeding tray open. When the test bird came down to feed, it was startled by releasing the cord and closing the lid on the feeding tray. After startling the test bird, the lid was immediately returned to its upright position allowing access to the feeding tray again. During the test, all test birds were accompanied by a "companion" bird. These companion birds were chosen randomly from the pool of birds available such that companion birds were not used more than once. A bird was always a test bird first before being a companion to others. After entering the aviary, both birds would normally fly to a perch. We measured the time taken for the test bird to come down to the feeding tray and take seed (time

to first seed). We expected this first latency to reflect a "novelty effect" as the experimental set-up (but not the aviary) and being in the company of just one other bird was new. This first phase of the experiment reduces the novelty effect and familiarizes the birds with the new situation. In the second phase, we measured the time it took the test bird to return to the feeder for a second time. At this stage, we startled the bird without allowing it time to feed. In the third phase, we measured the time it took the bird to return to the feeding tray after being startled. Following the study of van Oers and colleagues (2004), we called this the "startle latency" and we expected the "startle latency" to reflect a predisposition to risk-taking behavior. During this test, all other birds were kept in an adjacent cage (wooden shed) out of visual contact. The startle test is also referred to as the risk-taking test in this study as in others (Drent et al., 2003; van Oers et al., 2004).

Statistical analyses

The data collected were analyzed using a general linear model (GLM), univariate analysis of variance, to test for any significant effect of corticosterone line, sex, bird color (phenotype), sex of companion and corticosterone titre value (measured during selection procedure) on both exploratory behaviors, its sum of scores and number of feeders visited and startle tests (first seed latency and startle latency). On all analyses to control for the possible effect of the two replicate lines, we added "replicate" as a random effect in the model. A linear model including all variables was produced, with subsequent stepwise deletion of least significant variables, to give a final minimum adequate model. Residuals were checked for normality and data were transformed prior to re-running the analyses when appropriate. As the startle test was run over 10 days and a season or day effect was expected, data (as in numbers from 1 to 10) were entered in the model as a covariate. The minimum adequate model for all analyses included replicate and line as these variables were deemed to have biological meaning. For the same reason for the startle test only, the minimum adequate model also included date and the sex of the companion bird. For clarity, we also performed the same GLM test for birds in the company of a male companion and a female companion separately. The statistical analyses were performed in SPSS (version 11.0 for Windows XP). The results of all analyses are considered significant at the two-tailed probability of 0.05 or lower and they are not shown if probability higher than 0.05.

Ethical statement

During this study, we have adhered to the animal welfare standards of the UK Home office and all tests were carried out after local ethical review. Blood samples for corticosterone were carried out only at the selection procedure which is published elsewhere (Evans et al., 2006) and licensed by the UK Home office.

Results

Exploratory and novelty test

(i) Novel environment and novel objects

The minimum adequate model for novel environment, novel objects and sum of scores from these two tests showed no effect of either corticosterone line, corticosterone titre, sex or replicate.

(ii) Number of feeders visited

The results from the GLM show that the corticosterone titre had a marginally significant effect on the number of feeders visited (Table 1). The interaction between corticosterone titre and corticosterone line was also significant indicating that within lines, titre affected number of feeders visited differently. Examination of the parameters table shows that the effect of corticosterone titre was significantly positive in the low corticosterone line, i.e. higher titre individuals within this group

Table 1
Significant variables in the minimum adequate model of number of feeders visited

Variable	F (df)	P	Parameter estimate (SE)
Constant	92.949 (1,42)	<0.001	2.270 (0.455)
Corticosterone titre	4.075 (1,42)	0.050	−0.010 (0.036)
Corticosterone titre × corticosterone line	3.323 (1,42)	0.046	
Titre × low			0.176 (0.071)
Titre × control			0.017 (0.047)
Titre × high			0.000 (0.000)
Sex	3.472 (1,42)	0.069	
Female			−0.511 (0.274)
Male			0.000 (0.000)

Table 2
Significant variables in the minimum adequate model of log$_{10}$(first seed latency) for (A) all data pooled and (B) test birds in the company of females

Variable	F (df)	P	Parameter estimate (SE)
(A) Constant	2741.69 (1,23)	0.004	3.350 (0.422)
Corticosterone line	3.857 (2,23)	0.036	
Low			0.535 (0.212)
Control			0.534 (0.214)
High			0.000 (0.000)
Sex companion	5.148 (1,23)	0.033	
Female			−0.601 (0.318)
Male			0.000 (0.000)
(B) Constant	24.95 (1,18)		2.181 (0.266)
Corticosterone line	4.92 (2,18)	0.020	
Low			0.645 (0.245)
Control			0.624 (0.222)
High			0.000 (0.000)

visited more feeders, but was not significantly different from a line of zero slope within the other two lines, i.e. increases in titre did not add any more feeders to high or control line individuals (Fig. 1).

Risk-taking or startle tests

(i) First seed latency

The time taken from entering the home cage and going down to a feeder for the first time had to be transformed for analysis. The minimum adequate model (Table 2A) shows that log$_{10}$(first seed latency) is affected by both corticosterone line and the sex of companion bird. An investigation of the parameter estimates shows that high line birds take a shorter time to come down to feed for the first time. If the test bird is housed with a female, the time taken to go down is shorter than if the test bird (either sex) is housed with a male (Fig. 2B). Because of this effect of companion sex, we also tested birds in the company of males or females separately. If a bird was tested in the company of a female, there was an effect of corticosterone group with high

corticosterone birds coming down faster than all the other birds (Table 2B, Fig. 3A). There was no effect of any of the variables tested on the time taken for the first seed when birds were tested with a male.
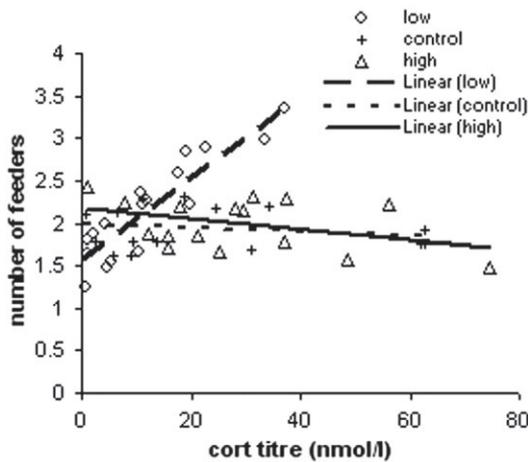

Fig. 1. Relationship between number of feeders visited (one measure of exploratory behavior) and corticosterone titre (nmol/L). Different symbols and trend lines denote the corticosterone lines (high, control and low). All points are predicted values from the final GLM model for each individual bird. The interaction between corticosterone line and corticosterone titre is significant ($P<0.05$), indicating that within lines, titre affected number of feeders visited differently.
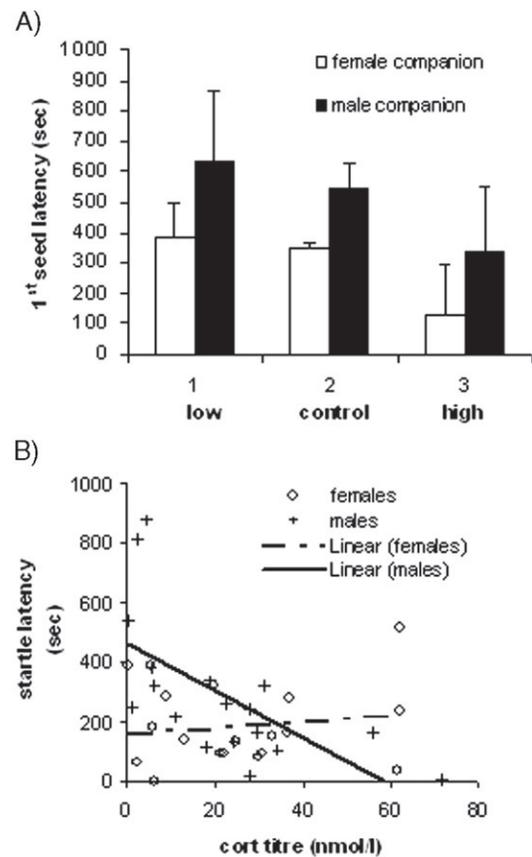

Fig. 2. (A) Mean time to first seed (first latency, in seconds) per corticosterone line and sex of companion. Both corticosterone line and sex of companion significantly affect first latency ($P<0.05$). (B) Relationship between time taken to return to seed after startle (startle latency in seconds) and corticosterone titre. Symbols and trend lines denote sex of test bird. Values for females and males are predicted values from the final GLM model. The interaction between corticosterone titre and sex is significant ($P<0.05$).

(ii) Startle latency

The time taken to return to feed after being startled was dependent on the corticosterone titre of the test bird (Table 3A) with parameter estimates showing that birds with higher corticosterone titre returned to the feeder faster than birds with low corticosterone (Fig. 2B). The main effect of line was not significant in this model. In addition, there was a significant difference between the two replicates with birds from replicate 1 taking much longer to return after being startled irrespective of corticosterone. The day on which the test was carried out also affected the time taken for birds to return after the startle.

When the data were split by sex of companion bird, there was no effect of any of the variables tested on the time taken to return to feed after the startle when birds were housed with males. However, when tested with a female companion, and when compared to birds from the high corticosterone group, birds from the low and control groups were affected by corticosterone titre negatively, so within those groups the higher the corticosterone titre the shorter the time to return after a startle. In relation to males, corticosterone titre affected females
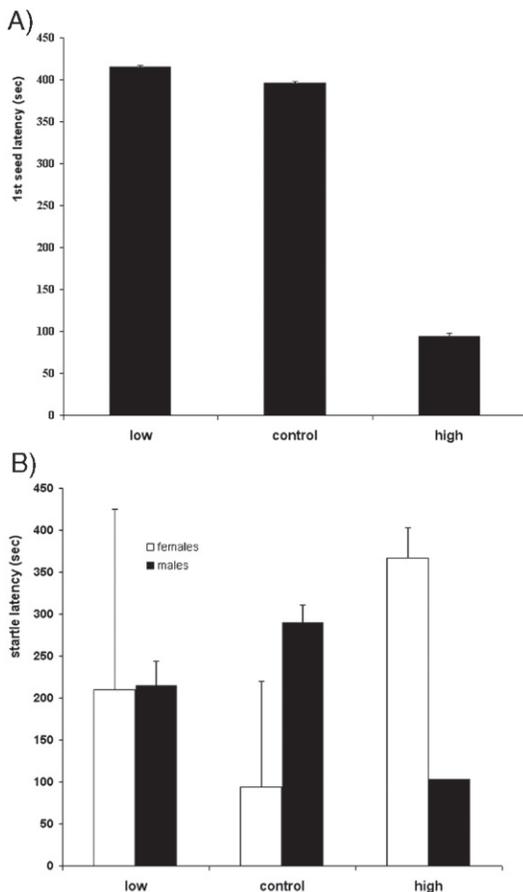

Fig. 3. (A) Mean time to first seed (first latency in seconds) per corticosterone line for birds tested with a female companion. (B) Relationship between mean time taken to return to seed after startle (startle latency in seconds), corticosterone line and sex of test bird for those birds in the company of a female. Interactions between sex of test bird and corticosterone titre are significant (P<0.05).

Table 3
Significant variables in the minimum adequate model of startle latency (or time to return to feeder after being startled) for (A) all data pooled and (B) test birds in the company of females

Variable	F (df)	P	Parameter estimate (SE)
(A) Constant	18.227 (1,24)	0.095	90.246 (183.408)
Corticosterone titre	5.720 (1,24)	0.025	−10.046 (4.200)
Date	4.945 (9,24)	0.001	
Day 4			660.668 (185.407)
Day 8			521.451 (192.401)
Replicate	4.637 (1,24)	0.042	
1			139.796 (64.917)
2			0.000 (0.000)
Sex	4.156 (1,24)	0.053	
Female			−157.101 (77.061)
Male			0.000 (0.000)
(B) Constant			−42.064 (152.614)
Corticosterone line	7.251 (2,12)	0.009	
Low			762.132 (210.858)
Control			512.370 (167.265)
High			0.000 (0.000)
Sex	9.752 (1,12)	0.009	
Female			0.448 (3.123)
Male			0.000 (0.000)
Corticosterone titre × cort line	6.416 (2,12)	0.013	
Titre × low			0.451 (3.140)
Titre × control			0.390 (2.772)
Titre × high			0.000 (0.000)
Sex × titre	8.796 (1,12)	0.012	
Female × titre			68.650 (23.148)
Male × titre			0.000 (0.00)

positively, i.e. females with higher corticosterone titre took longer than males to return to the feeder.

Relationship between measurements

The Spearman's correlation between the score from the novelty tests (environment and objects) was not significantly different from zero ($r_s = 0.004$, $n = 57$, $P = 0.912$).

Correlation (Pearson's) between first seed latency and startle latency in this study was higher (but non-significant) than correlation between number of feeders and first seed latency ($r_p = 0.216$, $n = 39$, $P = 0.094$; $r_p = 0.132$, $n = 34$, $P = 0.229$, respectively). If data are divided for birds with a male companion and with a female companion then the Pearson's correlation between number of feeders and first seed latency is high and significantly different from zero for birds with a male companion only ($r_p = 0.718$, $n = 14$, $P = 0.002$ and $r_p = 0.007$, $n = 18$, $P = 0.489$, for males and females respectively). This suggests that when in presence of males they behave in the same way as in a non-social situation in a new environment.

Discussion

This study has used selective breeding to test if exploratory behavior and risk taking is related to selected levels of circulating corticosterone. These birds were selected to have high, control and low levels of peak circulating corticosterone following a

manual restraint stress (Evans et al., 2006). The sub-sample of birds used in this study showed the same significant difference in peak corticosterone as measured during selection for generation 5.

Novelty tests

In the novelty tests, we used novel environment and novel objects to test for exploratory behavior. An interaction between corticosterone group and titre revealed that number of feeders visited (one measure of exploratory behavior) increased with corticosterone titre but only in the low corticosterone line birds. The data suggest that beyond about 40 nmol/L, there was little additional effect of corticosterone titre on the exploratory behavior of the birds in this particular test. Personality studies have shown that active individuals respond to stressors with lower HPA axis reactivity resulting in reduced increase in plasma glucocorticoid levels when compared to passive individuals (Koolhaas et al., 1999; Wingfield, 2003). This interpretation supports our results. By using selective breeding, we were able to show that birds that produce a low corticosterone peak response also show an increase in one measure of exploratory behavior, i.e. they show an active coping style. For selectively bred rainbow trout, low responding fish (low cortisol line) exhibited a proactive/active coping style while the high responding fish (high cortisol line) exhibited a reactive/passive coping style in some tests in unfamiliar environments (Øverli et al., 2002; Schjolden et al., 2005). However, what is interesting about our study is that increments in corticosterone are directly linked with increases in activity in the selected low corticosterone line due to our experimental approach and the model used for the data analysis (corticosterone titre as a covariate), thus introducing the notion of "continuity" for both physiological trait and behavior.

The sum of scores measure of exploratory behavior was not affected by either corticosterone line or titre, sex of the bird or replicate and neither were the two scores of the novel objects tests. As the exploratory and risk-taking tests performed here were designed for great tits (Drent et al., 2003; van Oers et al., 2004), we believe that the low score for the novel objects test may have been due to the fact that zebra finches are highly social birds and do not respond well to being in social isolation. In addition, results suggest that the time span used for the test might not have been enough for single birds to approach unfamiliar objects (van Oers et al., 2005) but long enough to get closer to more familiar objects such as the feeding troughs. Sudden social isolation has been suggested as the cause for behavioral inhibition in other personality studies using highly social species such as chickens (Jones et al., 1995). For that reason, a "companion bird" was included in the aviary for the two startle tests. For great tits, focal birds in general took less time to take the first worm (first seed in this study) when a companion was present (van Oers et al., 2005). We can only speculate that the inclusion of companion bird in the novelty tests might have encouraged test birds to approach unfamiliar novelty objects in the time given for the test. Differences between familiar and unfamiliar set-ups have been shown to produce divergent results in at least one other study (Schjolden et al., 2005).

Risk-taking tests

In the first latency part of the startle test, we were able to show that birds from high corticosterone lines went down to feed faster than all other birds. Interestingly, the sex of the companion bird affected how quickly birds went down to the feeder. This difference seems to be produced because male companions interacted more with the test bird than female companions. We suggest that this interaction may have distracted the test birds from feeding. This can be seen in the lack of significant results for the risk-taking variables when test birds are in company of males.

There was an effect of corticosterone titre on the time taken to return to the feeder after being startled. This result was in agreement with the first latency result as the higher the corticosterone titre the shorter the return time after the startle. Not surprisingly, considering the experimental set-up, correlation between first latency and startle latency in this study was higher (but non-significant) than correlation between number of feeders and first latency.

The low correlations between first latency and both the number of feeders and the sum of scores is surprising as the three tests should reflect responses to novelty. However, one has to consider that even though the tests are supposed to be measuring an individual's response to novelty (van Oers et al., 2004), they differ in at least three details: (1) the presence of food as reward in the startle tests, (2) the place where the test were performed (i.e. number of feeders was performed in the test cage and both startle tests in the aviary) and (3) the presence of a companion bird in the startle test. The fact that the first latency was a test in response to food might explain the readiness of high corticosterone birds to go down to feed first and the apparent lack of agreement between results for the novel objects and the startle tests. An acute elevation of corticosterone levels tends to switch on what is called the emergency life history stage and tends to increase foraging behavior in many birds (Wingfield, 2003; Wingfield, 2002). It is chronic elevation of corticosterone that tends to suppress foraging and reproductive behavior (Wingfield, 2003). The birds used in this study were selected to respond to handling stress by producing higher plasma corticosterone and do not necessarily show a chronically high level of corticosterone (Evans et al., 2006). In addition, it has been shown for peacocks that high corticosterone concentrations are associated with behavioral traits in males showing a dominant profile (Mateos, 2005) and as dominance is more likely to be displayed when food is available it is possible presence of food could affect results. The possible effect of the "environment" used for the tests can be seen in the fact that correlations between tests held in the same "arena" are higher (but non-significant) suggesting that previous knowledge of the environment tends to influence results. In the study of van Oers and collaborators (2004) the same "test environment" effect occurred: wild great tits showed a high correlation between exploration and risk-taking variables but a low correlation with boldness, which was performed in another "test environment". Rainbow trout selectively bred to show high and low levels of cortisol showed differences in behavior when in unfamiliar

environments but not when in familiar ones (Schjolden et al., 2005). For behavior to be repeatable and correlated over time and across situations, standardizing the "test environment" might be important if we are to produce repeatable and consistent results (Kralj-Fišer et al., 2007). This can be seen in our study in the high correlation between the number of feeders (a measure of exploration) and the first latency (another measure of exploration) for the data set for birds in the company of males: test birds in the company of males behaved as if in an unfamiliar arena and in isolation.

The inclusion of a male companion in the startle tests influenced the time taken for test birds to approach the feeder initially (first latency) as a male companion was more likely to distract the test bird from going down to feed. When the data are split for companion sex and tested again, it can be seen that in the presence of a male no effect of corticosterone group or titre is found. The data set for birds tested in the presence of a female companion shows that high corticosterone birds behave in an active/proactive coping style.

In general our results show that increases in corticosterone tend to result in raised exploratory and risk-taking behaviors, i.e. an active or bold personality. This result is surprising because the personality literature suggests that high HPA axis response (i.e. high peak plasma corticosterone) should be a characteristic of passive or less exploratory animals (Koolhaas et al., 1999). The key difference between our study and most others that have investigated the relationship between hormones and behavioral syndromes is that in our study we used birds that had been selected to have differing levels of plasma peak corticosterone and then investigated differences in behavior. Studies on selective breeding where lines with different levels of hormone are produced, such as in this study, are more likely to produce the desired link between physiology and coping styles. Similar relationships between behavioral reactivity and physiological stress coping assessed by HPA axis reactivity were found in selected fish (Øverli et al., 2002, 2004; Schjolden et al., 2005; Summers et al., 2005). Other studies have concentrated into dividing individuals into two separate behavioral categories (e.g. active v passive, low feather pecking, high feather pecking, etc.), selectively breeding them and then looking at how they perform in different situations or what their glucocorticoids levels are, i.e. selected chicks (Korte et al., 1997), selected great tits, (Carere et al., 2003), selected Japanese quail (Cockrem, 2006). These studies have also gained some success in linking HPA axis reactivity with behavior and physiology. On the other hand several studies of unselected animals failed to show this relationship (Cockrem, 2006; Palme et al., 2005; Rödel et al., 2006).

Traditionally studies on coping styles have tended to report differences in physiology between the extremes of coping styles without necessarily characterizing the relationship linking hormone levels and behavior (Carere et al., 2003; Korte et al., 1997; Pfeffer et al., 2002), both of which will tend to vary continuously. One reason might be that coping styles are often portrayed as bimodal (Koolhaas et al., 1999) and not continuous traits, although the behavior underlying the coping style will vary in a continuous way (i.e. time explore, time to return, etc.). Selective breeding for hormonal levels therefore represents a change in approach as

differences in physiology due to selective breeding can be linked to characterized coping styles (Schjolden et al., 2005).

Studies to date have tended to show that it is individuals with passive or shy personalities which seem to produce more corticosterone during mild stress such as restraint (Korte et al., 1997) or a social challenge (Carere et al., 2003). Intraspecifc aggression (and consequent social defeat) has been reported as one of the most severe stressors in terms of neuroendocrine activation (Koolhaas et al., 1997). This has caused many researchers to overlook the possibility that most dominant and active males during breeding season have higher glucocorticoids concentrations (Mateos, 2005). So, results can be dependent on season or whether tests involve food or social isolation, for example young greylag geese that excreted higher levels of fecal corticosterone later showed short latencies to approach a task and showed innovation at the feeder while the birds secreting low levels of fecal corticosterone became "food scroungers" (Pfeffer et al., 2002). Pfeffer and collaborators concluded that the high corticosterone "food producers" showed characteristics of both active or passive coping styles as short latencies are typical of an active style while high reactivity to environmental stimuli is regarded as typical of passive coppers (Koolhaas et al., 1999; Pfeffer et al., 2002). In chickens from low and high feather pecking lines (LFP and HFP), plasma corticosterone response to a mild restraint was higher in the LFP line than in the high pecking line birds (Korte et al., 1997, 1999). However, LFP chickens showed no behavioral inhibition in open field tests when in social isolation as compared to HFP hens (Jones et al., 1995; Korte et al., 1997). This suggested to the authors that high corticosterone LFP birds had an active coping style. For rainbow trout, swimming activity was higher in LR (low cortisol) fish immediately after they were transferred to the open field aquaria; however, these fish spent longer period within the cage before exiting into the "stream channel". LR fish were therefore considered proactive/active in style while HR were considered reactive/passive. However, HR (high cortisol line) attacked the intruder more frequently than the LR individuals.

The data to date suggest that a simple characterization of active and passive coping styles and its physiological differences between individuals and across species is still under way. One possible reason is that acute elevated plasma corticosterone may either promote an active response or is permissive to escalated activity (Summers et al., 2005), depending upon time (Summers and Winberg, 2006). For example, during breeding, levels of glucocorticoids (corticosterone and testosterone) are present in more dominant and active males peacocks who can withstand the possible costs of immunosuppression (Mateos, 2005). Another possible reason why this may be so difficult is that personality almost by definition is bimodal—active or passive, exploratory or shy. In reality the behaviors used to define personality are continuous (the time taken to return to a feeder), similarly hormone titres are a continuous variable (nmol L^{-1} corticosterone). It therefore becomes unclear where a line must be drawn across a continuously distributed trait to divide it into conveniently categorical personality divisions. While characterization of behavior and physiology is not clear cut when lines are selected for behavior, selecting for physiology might be the way forward.

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