

## RESEARCH ARTICLE

### Coping with novelty and stress in free-living house sparrows

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Accepted 10 November 2010

#### SUMMARY

Individuals respond differently to stressors and it has been suggested that stress responses are related to coping styles (consistent individual differences in behavior and physiology). We studied behavioral responses to a novel object and corticosterone response to stress during chick rearing in free-living female house sparrows (*Passer domesticus*). To prevent mates from influencing each others' behavior, we removed the males temporarily from nests and tested the females the following day either with a novel object placed on the nest box or as control. The two groups differed only in behaviors that were *a priori* defined as responses to the novel object (latency to first feeding, time spent near the nest, and inspecting the novel object by hovering in front of it) indicating that mate-removal *per se* had no effect on female behavior. Based on these variables, females' coping behaviors were categorized as 'bold', 'inquisitive' or 'shy' by discriminant analysis. Baseline corticosterone, measured on the day following the novel-object or control test, was not related to any measure of coping. Stress-induced corticosterone, however, was negatively related to number of hoverings in front of the nest (a measure of explorativeness) and accordingly differed between the behavioral coping categories, with 'inquisitive' birds having the lowest stress response. We propose that the relationship between physiological stress response and behavioral response to novelty (a component of personality or coping style) may be more complex than previously suggested, and individuals cannot always be unambiguously categorized along a single personality axis.

Key words: personality, coping style, corticosterone, stress response, neophobia, novel-object test, *Passer domesticus*.

#### INTRODUCTION

Individuals of many animals from humans to fish and even arthropods differ in how they react to environmental stimuli such as novelty, risk or sociality. The concept that non-human animals exhibit consistent individual differences very similar to human personalities has received growing research interest by behavioral ecologists for the last decade (Groothuis and Carere, 2005; Sih et al., 2004; Réale et al., 2007). These individual differences have been termed, temperament, coping style, personality, behavioral syndromes or behavioral tendencies, with several definitions proposed that "describe the phenomenon that individual behavioral differences are consistent over time and/or across situations" (Réale et al., 2007). The study of animal personalities already indicates that behavioral tendencies are remarkably similar to traditional individual traits in that they show genetic variation and fitness consequences (for a review, see Réale et al., 2007) (Smith and Blumstein, 2008).

Meanwhile, evolutionary endocrinologists have delved into the physiological mechanisms by which animals cope with environmental challenges, with a plethora of studies focusing on the glucocorticoid stress response (for a review, see Landys et al., 2006). Glucocorticoid hormones released in response to severe unpredictable noxious stimuli are thought to be the main factors mediating the physiological stress response (for a review, see Wingfield, 2003). Glucocorticoid levels also demonstrate individual variation and consistency (e.g. Cockrem and Silverin, 2002; Cockrem et al., 2009), heritability (Evans et al., 2006), phenotypic

plasticity (Lendvai et al., 2007) and fitness consequences (Romero and Wikelski, 2001; Blas et al., 2007), and evolve as adaptive traits (Bókony et al., 2009).

Recently, both theory and empirical research began to explore possible links between animal personalities and the stress response. The Hawk–Dove model (Korte et al., 2005) predicts that bold, fast, aggressive individuals are less responsive to stressors than shy, slow, passive individuals. Such a variation may be maintained by spatial or temporal variability in selection pressures, e.g. bold behavior and low stress responsiveness may be adaptive in constant environments whereas shy individuals with high stress responsiveness may be more successful in variable environments (Cockrem, 2007). Recent models suggest that behavioral tendencies arise by individual differences in the 'solution' of life-history trade-offs (Wolf et al., 2007; Biro and Stamps, 2008) that are mediated by physiological systems such as glucocorticoid and other hormones (Biro and Stamps, 2008; Lendvai et al., 2007). Accordingly, a number of case studies support the idea that the stress response may be a part of the coping style of birds (reviewed by Cockrem, 2007). Other studies have produced mixed results (Overli et al., 2002; Veenema et al., 2003; Marin and Satterlee, 2003; Schjolden et al., 2005; Martins et al., 2007; Koolhaas et al., 2010). These studies have been performed mostly on species subjected to artificial selection. Although such studies have the benefit of experimentally controlled conditions, the validity and generality of their results to natural populations is open for debate (Lambrechts et al., 1999). Artificial environments often expose animals to new selection regimes compared with their wild

counterparts, and even when experimenters do their best to control for all sources of bias, captivity and human proximity might influence the very traits they are trying to investigate. For example, selection studies of corticosterone (the main avian glucocorticoid) found directional ‘evolution’ of corticosterone levels over a few generations in the *control* lines, an effect that appeared comparable in magnitude to the artificial selection pressure applied to the selected lines (Evans et al., 2006). Similarly, the behavior of animals may also be altered inadvertently by selective breeding, e.g. different strains of laying hens differ in fearfulness (Fraisse and Cockrem, 2006). Furthermore, artificial selection could break off naturally occurring covariations between traits or produce differences that are not observed in the wild (Lambrechts et al., 1999; Evans et al., 2006; Moretz et al., 2007). Therefore, it is important to explore how behavioral tendencies and physiological coping styles are related in non-domesticated, free-living animals.

In this paper we report a field study of naturally breeding house sparrows, *Passer domesticus* L., to investigate the relationship between behavioral and hormonal differences in coping styles among individuals. To assess the birds’ coping styles, we investigated their behavioral responses to a novel object and their corticosterone response to a mild stressor, the standard capture–restraint protocol. In captivity, house sparrows exhibit considerable individual variation in object and food neophobia (Martin and Fitzgerald, 2005), and these two traits are correlated and show individual consistency (V.B., A. Kulcsár and A. Liker, unpublished data), indicating that they constitute an aspect of personality in sparrows, similarly to other bird species, where object neophobia was found to be consistent between individuals (e.g. Boogert et al., 2006; Stöwe et al., 2006b). The corticosterone response is well studied in house sparrows and varies across individuals with sex, season and brood value (Breuner and Orchinik, 2001; Romero et al., 2006; Lendvai et al., 2007; Lendvai and Chastel, 2008). To minimize the latter effects in our analyses, we restricted our study to breeding females and took into account the time of season and various measures of offspring value. Based on the hypotheses presented above (Korte et al., 2005; Cockrem, 2007) and the results from other bird species (Carere et al., 2003), we predicted that behavioral responses and stress reactivity should be correlated, with the boldest individuals being the least thorough explorers and having the lowest increase in corticosterone levels in response to stress.

## MATERIALS AND METHODS

### Study species

The study was carried out between April and July 2007 on a free-living population of house sparrows that breed in nest boxes in Chizé (46°08′50″N, 0°25′29″W), France. A large proportion of the adults used in this study were first captured either in previous years or during the pre-breeding period using mist nets and marked with a unique metal ring and color combination. Nest boxes were monitored at least every third day to determine the date of clutch initiation and clutch size. Starting 9 days after the clutch completion, nest boxes were checked at least once every day to determine the exact date of hatching (day 0). Brood sizes in this study ranged from three to five chicks.

### Protocol

The aim of the study was to investigate the relationship between the stress response of breeding birds and their behavioral response to a novel object in the field. One difficulty with measuring behavioral response in breeding birds in the field is that the behavior

of one parent may influence the behavior of its partner. Therefore, we used short-term removal of one parent to assure the statistical independence of the individuals’ behavioral response in the test situation. Hence, our study consisted of four phases: (1) observation of ‘baseline’ behavior of the parents, (2) temporary removal of the male, (3) observation of females in the novel-object test and (4) measuring the stress response of the females.

In each focal nest, we observed the baseline behavior of the parents on day 7 (i.e. when the chicks were 7 days old). Observations lasted 60 min and were started in the morning hours, approximately 3 h after sunrise, because 1-h observations in this time period were found to best predict the sparrows’ parental behavior during the whole day (Schwagmeyer and Mock, 1997). We observed the nest box and its vicinity within a radius of ca. 2 m from a car parked 30–50 m away. We recorded the time when the parents were in sight and their behavior (see details below).

On day 7, after the observations, we captured the male parent using a trap installed inside the nest box, and took him into captivity (for details, see Lendvai and Chastel, 2008). Males were housed in individual cages (30×40×50 cm; length × breadth × high) where food and water were provided *ad libitum*. Cages were placed indoors at ambient temperature and natural lighting conditions, and they were isolated from other sparrows. After capturing the male, we ringed the chicks and measured their body mass ( $\pm 0.1$  g).

The next morning (on day 8), we recorded the behavior of the female parent in a behavioral test (control or novel-object test; see details below). Immediately after the observation, the male parent was released from captivity. No male was observed to desert the nest after being released from captivity. Whenever it was possible, we timed the capturing of the males for the late-afternoon hours on day 7 to minimize the effect of the mate-removal on the females’ behavior. Therefore, the mean time elapsed from capturing the male until the behavioral test of the female was  $15.14 \pm 0.28$  h ( $\pm$  s.e.m.; including on average 8.85 h of night time). To control for any potential effect of mate removal *per se*, we used a control group in which the male parent was removed on day 7 and the behavior of the female was recorded in the morning of day 8 without the novel object. Nests were randomly allocated to the novel-object or control groups. Note that in natural pairs, males may participate little, or not at all, in parental care, especially when the chicks are older; and females can raise their broods alone; so our removal of the male is unlikely to have triggered abnormal behaviors in the females.

On day 9, on average  $39.2 \pm 0.59$  h after the behavioral test, the female was also captured in the nest box. To measure the stress response of females, we used a standard capture–handling–restraint protocol (Wingfield, 1994). Immediately after capture, a small blood sample ( $\sim 100$   $\mu$ l) was collected from the brachial vein and the time required to collect the sample was recorded. Mean handling time was  $169 \pm 5.9$  s ( $\pm$  s.e.m.). Corticosterone levels measured at the initial bleeding were not related to handling time ( $F_{1,25} = 2.17$ ,  $P = 0.153$ ). Therefore, we assume that the corticosterone levels measured at capture were close to and representative of the circulating baseline hormone levels prior to capture. After collection of the initial (baseline) blood samples females were placed in cloth bags while the chicks were weighed again. Subsequent blood samples were collected from the females 30 min after the first bleeding (second sample), when corticosterone levels are known to be at their maximum for this species (Breuner and Orchinik, 2001; Lindström et al., 2005; Romero et al., 2006). Blood samples were kept on ice and centrifuged [5000 r.p.m. (1396 g), for 6 min] as soon as possible. Plasma was separated and stored at  $-20^{\circ}\text{C}$  until further analyses.

After the second blood sample was collected, the females' tarsus length, wing length and body mass were measured before releasing the birds.

#### Novel-object test

We measured the neophobia of the birds in a widely used test situation in which an unfamiliar but neutral object (which does not resemble any predator, competitor or conspecific) is presented to the test individual (Martin and Fitzgerald, 2005). We used, as the novel object, a juggling ball with a diameter of 8 cm that had four brightly colored parts (red, green, blue, yellow). The ball was fixed on the top of the nest box at the start of the observation. In the control group, the nest box was similarly approached at the start of the observation but no object was placed on the top. We had 18 novel-object nests and 11 control nests.

During the observations, we recorded the number of feeding visits per chick (henceforth referred to as feeding rate) and the time spent brooding [if individuals spent more than 1 min inside the nest box, they were considered to be brooding the chicks (see Chastel and Kersten, 2002)]. Furthermore, we recorded the following three variables as proxies for neophobia. First, we measured the time spent within 1 m of the nest box with food items in the beak (henceforth referred to as time spent nearby). This behavior is an indication of a behavioral conflict; it is often observed upon moderate disturbances that a parent arriving at the nest box to feed the chicks remains outside and waits until danger is passed. Second, we measured the latency to first feeding, i.e. the time elapsed from the start of the observation until the parent first entered the nest box. For individuals that did not feed their chicks during the observation, the latency was set to 61 min. These two variables probably reflect the risk evaluation ('boldness') of the individuals and the degree to which they consider the novel object threatening. Finally, we measured the number of times the focal bird hovered in front of the nest box. As most nest boxes were installed below the eaves of buildings, the birds could not see the ball from the gutter where they usually landed before entering the nest. Therefore, hovering in front of the nest box is probably a form of exploratory behavior by which the birds could observe the novel object from a distance. Only one bird landed next to the ball, whereas all birds hovered in front of it at least once. However, birds never showed hovering behavior when the ball was not present.

#### Hormone assays

Total plasma corticosterone levels were determined by radioimmunoassay at the Centre d'Études Biologiques de Chizé. Total plasma corticosterone was measured in samples (25 µl) after ethyl-ether extraction by radioimmunoassay using a commercial antiserum, raised in rabbits against corticosterone-3-(*O*-carboxymethyl) oxime bovine serum albumin conjugate (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Duplicate aliquots of the extracts were incubated overnight at 4°C with 9.25 MBq of [<sup>3</sup>H]corticosterone (Perkin Elmer, Courtaboeuf, France) and antiserum. The bound and free corticosterone was separated by adding dextran-coated charcoal. After centrifugation, the bound fraction was counted in a liquid scintillation counter. Total corticosterone was measured in one assay and the intra-assay coefficient of variation was 4.91% for five duplicates. The minimum detectable level of corticosterone was 0.52 ng ml<sup>-1</sup> (lowest measurement: 1.54 ng ml<sup>-1</sup>). [<sup>3</sup>H]corticosterone recovery from the extraction was 93.7%. Pooled plasma of different house sparrows produced a dose–response curve that paralleled the corticosterone standard curve. Mean recovery of added corticosterone was 108%.

#### Statistical analysis

All data processing and statistical analyses were performed in the R computing environment (R Development Core Team, 2006). Whenever possible, we used linear models ('lm' and 'glm' functions in R). Assumptions and fit of the models were checked by graphical diagnostic methods using 'plot.lm' function in R (Faraway, 2006). Since linear models of two continuous variables give identical results to Pearson correlation, we report the correlation coefficient for such bivariate analyses instead of *F*-values to show the direction of relationships.

Behavioral variables were calculated as the difference in the given variable between the test situation (novel object or control; day 8) and baseline behavior (day 7). Thereby we could analyze behavior irrespective of individual differences in activity levels or motivation (Martin and Fitzgerald, 2005). Positive values therefore indicate an increase in the given activity in the test situation compared with the baseline behavior. Prior to the analyses, all behavioral variables were log-transformed to improve model fit.

Because time spent nearby, latency to feed and number of hoverings was thought to reflect individual variation in response to the novel object, our *a priori* expectation was that the variance of these variables will be higher in the novel-object group than in the control group, and the data supported this expectation (Fig. 1). Owing to this heteroscedasticity, we could not use linear models to compare the two groups, therefore we analyzed the difference between the groups by using randomization tests ('twoPermutation' function in the DAAG package of R with 10,000 simulations).

Behavior and hormone levels of the females in the novel-object group and the growth of the nestlings were analyzed by linear models. To analyze chick growth, we used the difference in body mass, measured on days 7 and 9. In the analyses, we used the mean of the body mass and mass gain of the chicks per brood. Stress response was expressed as the stress-induced corticosterone levels in plasma (ng ml<sup>-1</sup>). To account for seasonal variation in the hormone levels, we used the date of female capture as covariate in the models. Ambient temperature recorded at the time of observations (to the nearest °C) did not affect the females' coping behavior (all *P*-values ≥ 0.2). Means ± s.e.m. and two-tailed probabilities are reported.

## RESULTS

### Test vs control birds

Before the behavioral tests, we found no difference between the novel-object group and the control group in body size (tarsus and wing length) and body mass of the adults (MANOVA, Pillai's trace=0.032, *P*=0.866, *N*=26), brood size (novel object: 3.44±0.17, control: 4.09±0.15, glm with Poisson distribution: *z*=0.88, *P*=0.38) and body mass of the chicks on day 7 (*t*<sub>1,25</sub>=0.11, *P*=0.912). On the test day, time spent nearby, latency to first feeding and number of hoverings differed significantly between the novel-object and control groups (randomization test, time spent nearby: *P*=0.002; latency to first feeding: *P*=0.028; number of hoverings: *P*=0.004; Fig. 1). However, the novel-object and control groups did not differ in either feeding rate or time spent brooding during the test (feeding rate: *t*<sub>1,27</sub>=0.67, *P*=0.507; brooding: *t*<sub>1,27</sub>=1.36, *P*=0.183). These results show that the removal of the mate *per se* had no significant effect on the females' overall behavior, because the novel-object group differed from the controls only in those behaviors that were thought to be responses to the novel object. Therefore the latter variables can be used to measure individual variability in neophobia as part of coping behavior. Finally, we found no difference between

the novel-object and control groups either in the baseline or the stress-induced corticosterone levels (Table 1).

### Behavioral coping styles

In the novel-object test, latency to first feeding was significantly and positively correlated with the time spent nearby (Pearson correlation,  $r=0.49$ ,  $P=0.039$ ,  $N=18$ ). The number of hoverings was

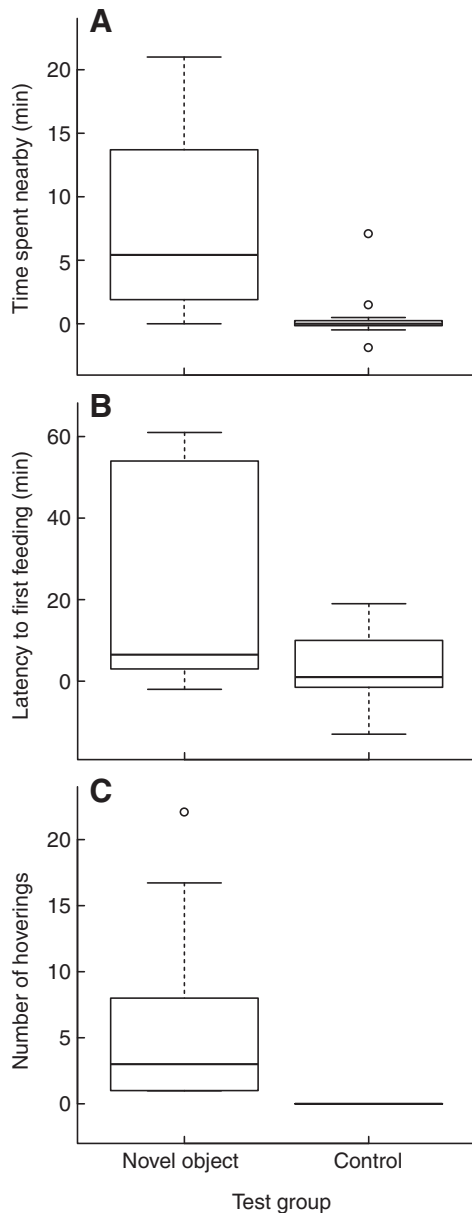


Fig. 1. Behavioral response of *Passer domesticus* to the novel object. The graphs show the difference in (A) time spent nearby, (B) latency to first feeding and (C) number of hoverings between the test day and the day before ('baseline' behavior) in the novel-object group and the control group. Positive values indicate an increase in the given activity in the test situation compared with the baseline behavior. For each box, the central line represents the median, and the bottom and the top of the box are the lower and upper quartiles, respectively. The whiskers extend to the lowest and highest observations, respectively, which are no more than 1.5 times the interquartile range from the box. The circles indicate outliers. Note that the median is zero for all three variables, indicating that the removal of the mate *per se* did not influence the birds' behavior.

Table 1. Corticosterone levels ( $\text{ng ml}^{-1}$ ) in the two test groups of *Passer domesticus*

Sample	Novel-object group	Control group	<i>t</i>	d.f.	<i>P</i>
Baseline	4.92±0.78	5.26±0.99	0.26	26	0.794
Second	27.91±1.65	32.35±2.92	1.35	24	0.189

Values are means ± s.e.m.

not related to either the latency to first feeding ( $r=-0.35$ ,  $P=0.157$ ,  $N=18$ ) or the time spent nearby ( $r=0.34$ ,  $P=0.169$ ,  $N=18$ ).

Based on these three variables, females were categorized into three groups: 'bold', 'inquisitive' and 'shy' (Fig. 2), and henceforth, these categories are referred to as coping styles. In the test situation individuals were categorized by comparison with the baseline behavior, as bold if they increased only slightly the time spent with food in the vicinity of the nest box (0 to 6 min), had low latencies (–1 to 7 min) and made few (1 to 5) hoverings in front of the novel object. Inquisitive individuals had higher but still rather low latencies (–2 to 25 min), but made more hoverings (11 to 22) and spent more time (5 to 16 min) with food in the vicinity of the nest box than bold individuals. Finally, we categorized individuals as shy if they hesitated long before feeding the chicks, or did not feed them at all, therefore they often remained with food in the vicinity of the nest box for long periods (2 to 21 min) and had high latencies (50 to 61 min), but rarely approached the novel object and therefore made few (1 to 8) hoverings in front of it.

We tested the validity of this categorization by a linear discriminant function analysis. The discriminant function analysis is a classification technique that generates a linear combination of variables which maximizes the probability of correctly assigning observations to pre-determined groups (Quinn and Keough, 2002). Therefore, by using discriminant function analysis, we could test whether the coping styles we defined, based on the behavior of the birds, reflected statistically different groups of individuals. The model produced two highly significant discriminant functions (Wilk's  $\lambda=0.009$  and 0.209, with eigenvalues 21.193 and 3.792; both  $P<0.001$ ), by which the individuals could be assigned to the three categories of coping styles with an accuracy of 100%. The grouping by the two discriminant functions is shown in Fig. 3.

Neither body mass of the chicks on day 9 or the mass change of the chicks from day 7 to day 9 differed between females with different coping styles (body mass:  $F_{2,14}=0.02$ ,  $P=0.998$ ; mass change:  $F_{2,14}=0.387$ ,  $P=0.685$ ).

### Hormones and neophobia

Baseline corticosterone was not related to any measure of neophobia (Pearson correlations, time spent nearby:  $r=-0.18$ ,  $P=0.487$ ; latency to first feeding:  $r=-0.07$ ,  $P=0.801$ ; number of hoverings:  $r=-0.14$ ,  $P=0.573$ ;  $N=17$ ) or to the coping style of the individuals ( $F_{2,14}=0.51$ ,  $P=0.612$ ).

Stress-induced corticosterone levels decreased with date (females of both the novel-object and control group included:  $r=-0.48$ ,  $P=0.006$ ), therefore we controlled for this seasonal effect in the following models by analyzing residual corticosterone levels obtained from a linear model with stress-induced corticosterone as response variable and capture date as an explanatory variable. Residual stress-induced corticosterone levels were not related to either the time spent nearby ( $r=-0.07$ ,  $P=0.797$ ) or the latency to first feeding ( $r=-0.04$ ,  $P=0.866$ ). However, the number of hoverings was significantly negatively related to the residual stress-induced corticosterone levels ( $r=-0.58$ ,  $P=0.017$ ; Fig. 4). Accordingly,

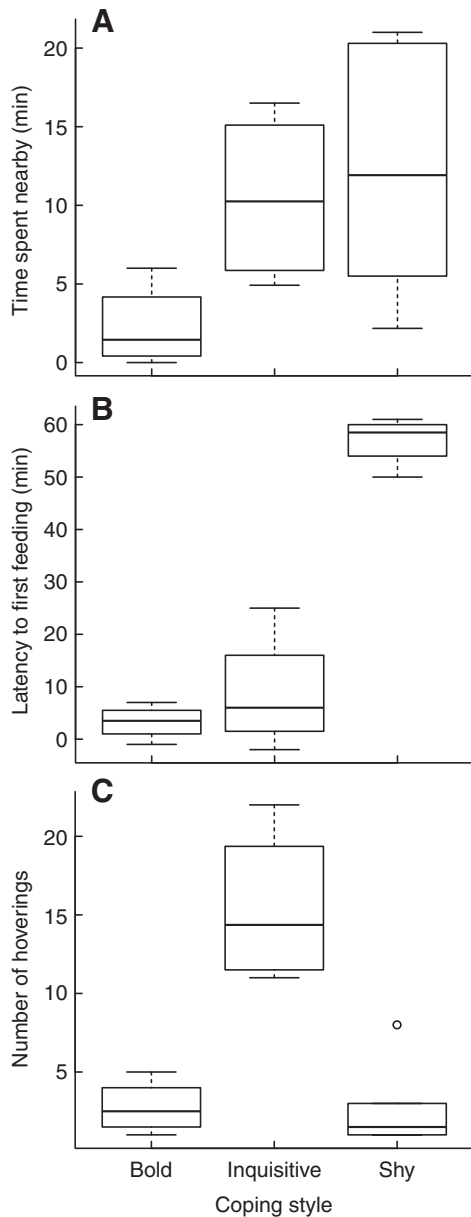


Fig. 2. Behavioral responses to the novel object in the three coping-style groups of *P. domesticus*. (A) Time spent nearby, (B) latency to first feeding and (C) number of hoverings (expressed as differences between the test day and the day before). Box plots as in Fig. 1.

residual stress-induced corticosterone also differed among the coping style categories, with inquisitive individuals having the lowest residual stress-induced corticosterone levels ( $F_{2,13}=4.49$ ,  $P=0.033$ ; Fig. 5).

The residual stress-induced corticosterone levels were not related to brood size (Spearman correlation:  $r_s=0.15$ ,  $P=0.707$ ), to the body mass of the chicks on day 9 (Pearson correlation: body mass:  $r=0.33$ ,  $P=0.202$ ) or to the mass change of the chicks between day 7 and 9 ( $r=0.42$ ,  $P=0.099$ ).

## DISCUSSION

Our novel-object test in the field was successful: we found large individual variation for all three behavioral variables that were used to reveal individual differences in coping styles. The behavior of

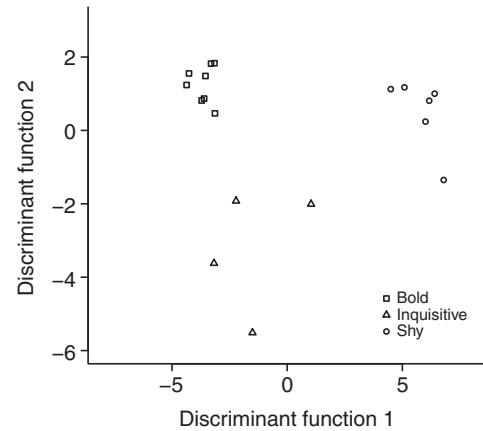


Fig. 3. Categorization of *P. domesticus* individuals' coping styles by two discriminant functions based on the three behavioral variables shown in Fig. 2.

the birds was apparently not influenced by removal of the mate *per se*, because: (1) test groups only differed in those variables that were *a priori* defined as behavioral responses to the novel object, and (2) the behavior of the control group did not change from day 7 to 8 (i.e. median for all differences was zero; see Fig. 1), indicating that the removal of the mate did not affect the fearfulness or risk evaluation of the individuals. This is probably due to the fact that we deliberately tried to minimize the time the male was absent. Therefore, the differences we observed among females in the test group are attributable to the ways they coped with the unexpected novel situation.

Some birds resumed chick feeding rapidly, others stayed away from the nest during most or all of the time the ball was on the nest box. A third group of the birds showed an interesting combination of behaviors: they returned to the nest quickly, but inspected the novel object many times by hovering in front of it. These behaviors probably reflect two components of coping: (1) risk evaluation (latency and time spent nearby; note that they were correlated), and (2) exploration (hovering). Although bold animals are usually less thorough explorers (Groothuis and Carere, 2005), we found no

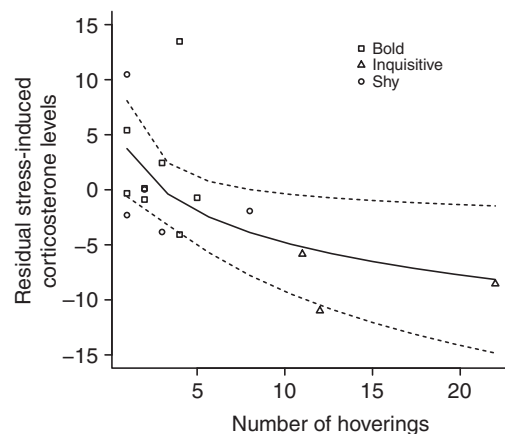


Fig. 4. Relationship between number of hoverings (log-transformed) and stress-induced corticosterone (date-corrected residuals) in *P. domesticus*. The solid line shows the fit of the model, the dashed lines show the 95% confidence intervals of the fitted line. Stress-induced corticosterone could not be measured for one shy and one inquisitive bird.

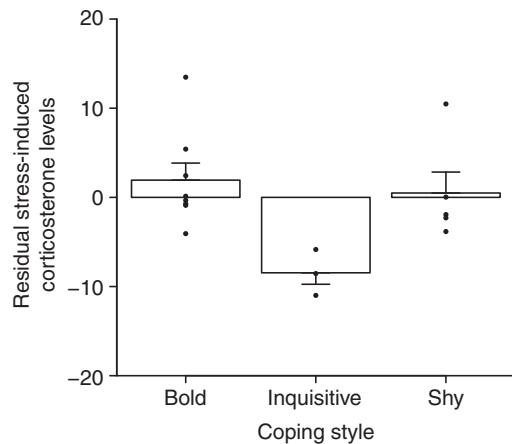


Fig. 5. Stress-induced corticosterone levels (date-corrected residuals) in birds (*P. domesticus*) with different coping styles (see Figs 1 and 2). The bars indicate the mean, the whiskers indicate the standard error of the mean. The dots show individual data points. Stress-induced corticosterone could not be measured for one shy and one inquisitive bird.

correlation between risk evaluation and exploratory behavior in this study. Coping styles in animals are typically depicted as two contrasting extremes [labeled as bold–shy (Career and van Oers, 2004); reactive–proactive (Koolhaas et al., 1999); slow–fast (Dingemanse and de Goede, 2004)], whereas human personalities are described along at least five different dimensions, out of which at least three seem to occur in non-human animals, too (Gosling and John, 1999). These personality dimensions, also referred to as behavioral axes (Sih et al., 2004) or temperament traits (Réale et al., 2007), need not be all correlated with each other (Cockrem, 2007; Martins et al., 2007; Brydges et al., 2008). For example, becoming an innovative greylag goose (*Anser anser*) requires apparently controversial temperament traits, i.e. boldness or proactive coping in terms of short exploring latencies and shyness or reactive coping in terms of high reactivity to environmental stimuli (Pfeffer et al., 2002), suggesting that innovativeness in geese resulted from a combination of two temperament traits, such as low neuroticism and high extraversion in the terms of human personality dimensions (Gosling and John, 1999). An alternative interpretation is possible in the light of an emerging concept, namely that stress reactivity and coping style may be two independent dimensions, with coping styles reflecting the quality and the stress reactivity reflecting the quantity of the response to environmental challenges (Koolhaas et al., 2010). According to this two-tier model, the innovative geese were neither shy nor bold, because the shy individuals are characterized by both high stress reactivity and reactive coping, whereas bold individuals are those with low stress reactivity combined with proactive coping behavior (Koolhaas et al., 2010).

Similarly, a combination of temperament traits characterized our inquisitive sparrows that showed relatively low fearfulness in terms of approach latency but, at the same time, seemed to explore the novel object more thoroughly than bold and shy birds. Stress-induced corticosterone levels differed between the three coping styles, but were related only to the exploratory behavior (hovering). One might argue here that the lower peak corticosterone levels of inquisitive birds may have been an artifact of the novel-object test if females that hovered a lot mounted a high stress response during the test that reduced their corticosterone secretion on the following day as

a result of negative feedback (Carere et al., 2003). Although we cannot rule out this explanation completely, we think it is unlikely because baseline corticosterone levels 1 day after the test did not vary with coping style or the number of hoverings, and neither baseline nor stress-induced corticosterone levels differed between the novel-object and control groups. Unfortunately, the inquisitive group had the smallest number of individuals. However, the discriminant function analysis showed that their behavior was statistically different from the other individuals. Even though a more equal distribution of the individuals among the groups would have been more helpful from a statistical point of view, the categorization of the individuals was based solely on their behavior in the novel-object test and the emergence of the distinct behavioral combination of the inquisitive sparrows was an important, albeit unexpected result of this study.

One might argue that this low sample size in the inquisitive group may bias the results owing to sampling error. Note, however, that all the residual corticosterone values of inquisitive birds lie beyond the range of data points from the bold and shy groups (Fig. 5). Also, the relationship between the number of hoverings and the residual corticosterone levels was significant (Fig. 4), and this analysis included all but two individuals from the novel-object group (for two individuals the stress-induced corticosterone data were missing). The validation of this model did not reveal highly influential points, and the result was robust whether or not we removed some of the most influential points, therefore it is unlikely that a few birds with high or low hovering activity may have disproportionately biased the results.

Thus, as far as explorativeness is considered a characteristic of shy or reactive personalities (Groothuis and Carere, 2005; Koolhaas et al., 2010), our result is inconsistent with the Hawk–Dove model (Korte et al., 2005). However, more time spent exploring a novel object may be interpreted as a manifestation of low neophobia and/or high neophilia (Greenberg, 2003; Stöwe et al., 2006a), in which case the low, rather than high, fearfulness of our inquisitive sparrows would match their low hormonal stress response as predicted by the Hawk–Dove model. Alternatively, according to the two-tier model of Koolhaas et al., the inquisitive individuals seem to match the category labeled as docile, which combines a reactive coping style (high exploratory behavior, intensive reaction to new environmental stimuli) and low stress reactivity (Koolhaas et al., 2010).

We found that risk-evaluation behaviors (latency to first feeding and time spent nearby) were not related to hormonal stress response, although the Hawk–Dove model suggests that this should be the case. Our result, however, provides further insights into the existing controversial findings on captive birds, showing that the relationship between behavioral and hormonal coping styles is not straightforward and depends on what and how is measured (Carere et al., 2003; Cockrem, 2007; Martins et al., 2007). One important difference between our study and previous ones is that our novel-object test was entirely realized in field conditions. Test environment, habituation and learning seem to affect the outcome of captive tests (Dingemanse et al., 2002), and authors often attribute the lack of correlation between certain aspects of coping styles to these shortcomings (van Oers et al., 2004; Schjolden et al., 2005; Martins et al., 2007). In our study, birds were tested only once in their natural environment, so individuals had no previous experience with the novel situation. We believe that the context dependence of the relationship between behavioral and hormonal coping styles is more than a mere artifact and deserves further investigation.

In parallel with individual differences in exploring the novel object, we found considerable variation among female sparrows in

their corticosterone response to a standard stressor. This supports the idea that hormonal response to stress is part of behavioral syndromes (Réale et al., 2007) and when responding to an environmental stressor, individuals may be 'constrained' by their personality. This seems to contradict our recent findings that individuals are able to flexibly modulate their stress response either up- or downwards as a physiological mediator for their adaptive allocation 'decisions' (Lendvai et al., 2007; Lendvai and Chastel, 2008). Individuals that are able to match their stress response to the actual circumstances may do better than less plastic individuals that produce lower or higher stress response than the optimal. However, limited plasticity may be adaptive if plasticity is costly or error-prone, e.g. if the environment is too variable to provide reliable information for making optimal decisions (Sih et al., 2004). Mounting a hormonal stress response is known to come with costs such as compromising the reproductive output (Wingfield, 2003; Landys et al., 2006; Bókony et al., 2009), therefore any wrong decision (e.g. a too high stress response) may jeopardize the individual's fitness. Repeatability and heritability of corticosterone levels (Cockrem et al., 2009; Wada et al., 2008; Evans et al., 2006) also imply their limited plasticity (Sih et al., 2004). Evidence for both individual flexibility and constraints in the stress response raise the interesting possibility that the plasticity of the stress response itself may be subjected to natural selection, which again parallels recent ideas about personality and behavioral plasticity (Dingemans et al., 2010).

In this study we implemented a novel-object test under field conditions to reveal individual differences in coping behavior. We found that using pre-defined behavioral variables, three distinct categories of coping styles emerged, and corticosterone response to stress differed between these coping styles, but was only correlated with explorative behavior (number of hoverings). Although our sample sizes are admittedly small, our behavioral and hormonal measures consistently support the recently realized concept that personalities of wild animals are more complex than just bold and shy (Moretz et al., 2007; Réale et al., 2007; Koolhaas et al., 2010).

#### ACKNOWLEDGEMENTS

V.B. and Á.Z.L. were supported by joint grants from the Centre Nationale de la Recherche Scientifique (CNRS) and the Hungarian Academy of Sciences; V.B. by an Eötvös State Grant from the Hungarian Scholarship Board (MÖB); and Á.Z.L. by the National Office for Research and Technology (NKTH; FR-33/2007) and Égide (17348RC), a postdoctoral grant from the Fyssen Foundation, and a grant from the Hungarian Scientific Research Fund (OTKA; PD76862). We are grateful to S. Dano, A. Lacroix and C. Trouvé at the CEBC for their technical assistance in hormones assays. We thank the anonymous referees for their valuable comments on an earlier version of this manuscript.

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