

Handling Stress Does Not Reflect Personality in Female Zebra Finches (*Taeniopygia guttata*)

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Although increasing attention is given to both the causes and consequences of variation in animal personality, the measurement of personality in captive or free-ranging individuals remains an issue. In particular, one important question concerns whether personality should be established from the existence of complex behavioral syndromes (a suite of correlated behavioral traits) or could be more easily deduced from a single variable. In that context, it has recently been suggested that handling stress, measured through breathing rate during handling, could be a good descriptor of personality, at least in passerine birds. The authors experimentally investigated to what extent handling stress was correlated with personality in female zebra finches (*Taeniopygia guttata*), as assessed from a suite of repeatable behavioral traits, including activity, exploratory behavior, neophobia, and reaction to startle. Although breathing rate was repeatable across individuals, it was not related to any behavioral trait, suggesting that it cannot be used to quickly predict personality, at least in zebra finches. Breathing rate during handling, in addition, was related to morphology, questioning the fact that breathing rate during handling reflects personality irrespective of individual state. The authors suggest that inference on global personality from a reduced number of traits should be performed with caution.

Keywords: behavioral syndrome, temperament, breathing rate, exploration, bootstrap

Interindividual differences in behavior (so-called “personalities”) have recently become of prime interest to ornithologists and behavioral ecologists (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Indeed, recent evidence has shown that personality

traits, such as neophobic or exploratory tendencies, can have fitness consequences (Smith & Blumstein, 2008) and can evolve through natural (Quinn, Patrick, Bouwhuis, Wilkin, & Sheldon, 2009) and sexual selection (Schuett, Tregenza, & Dall, 2010). It is interesting to note that some personality traits can be found to be related to each other at the population level, thus, forming a “behavioral syndrome” (Wilson et al., 2010). These correlations have several implications for behavioral studies. First, they suggest that different behaviors do not evolve independently but act as constraints toward each other, setting the stage for an integrative study of behavior (Sih, Bell, & Johnson, 2004). Second, these correlations, once established, can potentially enable experimenters to assess only a reduced number of traits to determine individual global personality (Fucikova, Drent, Smits, & van Oers, 2009). For instance, in great tits (*Parus major*), breathing rate after handling has been related to both exploratory tendencies and neophobia and used as an indicator of individual personality (Carere & van Oers, 2004; Fucikova et al., 2009). Exploratory birds had a higher breathing rate during handling (Fucikova et al., 2009), whereas bold individuals had a lower breathing rate (Carere & van Oers, 2004). These results suggest that breathing rate after handling might be used a proxy for personality in other bird species, given its ease of measurement in both field and lab conditions. Recent evidence, however, suggests that the assessment of personality may not always be reduced to the measurement of one single behavioral trait. For instance, David, Auclair, and Cézilly (2011) found that although neophobia, exploratory tendencies, activity,

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and reaction to a startle were related to each other within a broad behavioral syndrome in zebra finches (*Taeniopygia guttata*), obstinacy, while showing very high repeatability (i.e., within-individual consistency through time), was not related to any other personality trait (David et al., 2011). The use of a quickly assessable trait, such as breathing rate after handling, as a general proxy for personality in bird species then deserves further consideration. Therefore, following Fucikova et al. (2009), we investigated to what extent breathing rate that follows handling can predict personality in the zebra finch, while controlling for the effect of body mass and body condition on handling stress.

Method

Study Subjects

Forty-one wild-type female zebra finches (*Taeniopygia guttata*), in which personality had been assessed in a previous study (David et al., 2011), were kept in home cages (60 × 30 × 33 cm), by unisex groups of two or three birds, before the experiments. Zebra finches are small monogamous and gregarious passerine birds, widely used in behavior studies (Zann, 1996). Each individual was identified with an orange numbered ring (AC Hughes, Hampton Hill, England; size XF). Only females were used, as this study was part of a larger research program on the influence of personality on female sexual behavior. Our captive stock was kept in a breeding room where temperature was maintained at 22 ± 2 °C, and the photoperiod was 13:11-hr light–dark cycle (7:30 a.m.–8:30 p.m.). Birds were provisioned with millet seeds, cuttlebones, and water ad libitum. At the end of the experiment, birds were kept in groups of two to be used in subsequent studies. The experimental procedures were in agreement with the ethical requirements of the Université de Bourgogne, France.

Assessment of Personality Traits

Birds were food deprived for one hour before each trial to control for hunger levels. All tests were performed on individuals kept single. Activity corresponded to the number of movements performed in the home cage during a 10-min period. Neophobia was assessed from the tendency to feed near and to approach a novel object in the home cage. Exploratory tendencies were measured from the number of movements performed in a large unfamiliar cage (140 × 140 cm and 70 cm high) containing five artificial trees, each composed of four small branches. Reaction to a startle corresponded to the latency time to resume foraging in the home cage after a stressful event. All these personality traits have been found to be highly repeatable in zebra finches in a previous study (David et al., 2011). Detailed information concerning experimental apparatus and methodology can be found in David et al. (2011).

Handling Stress

Following previous studies conducted on small passerines (Carere & van Oers, 2004; Fucikova et al., 2009), we assessed handling stress from the intensity of breathing rate after being caught by an experimenter. All birds were kept single in their home cage. One experimenter (Y.A.) caught each bird in its own home cage and

measured the number of breast movements during 20 s as a measure of breathing rate (see Carere, Welink, Drent, Koolhaas, & Groothuis, 2001) while handling the bird on the back. Catching latency was also quantified as the latency time to catch the bird within its home cage. This procedure was repeated twice with a one-week interval between both trials, and both measures were averaged for each individual. All trials were performed at the same time of the day (6:00 p.m.) for all individuals in order to discard the potential effect of diurnal variation in behavioral activity or stress response. Handling-stress trials were conducted one day before personality tests began.

Morphometric Measures

All morphometric measures were taken twice on two different days by Y.A and two days before starting personality tests. Tarsus length was measured with a digital caliper to the nearest 0.1 mm. Birds were weighed to the nearest 0.01 g with an electronic balance (Ohaus, Scout Pro SPU202) at the same time of day for each individual. Values were then averaged for each bird. We determined body condition through the residuals calculated from the linear regression of \log_{10} (body mass) on \log_{10} (tarsus length; Schamber, Esler, & Flint, 2009).

Statistical Analyses

Consistency (*R*) of catching latency and breathing rate were assessed through Lessells and Boag's (1987) method. Differences between both handling trials were assessed through paired *t* tests, followed by the calculation of Cohen's *d* as a measure of effect size (Nakagawa & Cuthill, 2007). Statistics are reported with mean or *R* and 95% confidence interval (CI; Nakagawa & Schielzeth, 2010). Relationships between personality and breathing rate were tested by using Pearson's correlations. All statistical analyses were performed with JMP 5.0.1 (SAS Institute, Cary, NC).

Results

Overall, all measured behaviors were repeatable. However, whereas breathing rate was found to be related to body mass or catching latency, no relationship was found between breathing rate and any of the personality traits:

Mean catching latency was 6.8 ± 0.6 s (mean \pm 95% CI; *n* = 41). Catching latency and breathing rate were repeatable across both trials: catching latency, *R* = 0.31, 95% CI (0.02, 0.60), *F*(40, 81) = 1.90, *p* = .02; breathing rate, *R* = 0.66, 95% CI (0.47, 0.84), *F*(40, 81) = 4.80, *p* < .0001. Catching latency moderately predicted breathing rate during the first trial, *R*² = 0.14, bootstrap 95% CI (<0.01, 0.37), *F*(1, 40) = 5.52, *p* = .02, but not during the second one, *R*² = 0.04, bootstrap 95% CI (<0.01, 0.19), *F*(1, 40) < 0.01, *p* = .99. However, overall, variation in breathing rate in relation to catching latency did not differ between the two trials: analysis of covariance, *F*(2, 40) = 1.88, *p* = .17. Catching latency did not differ between trials, *t*(1,40) = -0.37, *p* = .71, *d* = -0.07, whereas breathing rate slightly increased from 66.2, 95% CI (64.5, 67.9) to 67.9, 95% CI (66.0, 69.7) breast movements between trials, *t*(40) = 2.37, *p* = .02, *d* = 0.04. The influence of personality on breathing rate was thus analyzed after pooling data from the two trials or considering each trial separately.

Average breathing rate was moderately predicted by body mass, $R^2 = 0.12$, bootstrap 95% CI ($<0.01, 0.32$), $F(1, 40) = 4.49$, $p = .04$, but not by body condition, $R^2 = 0.04$, bootstrap 95% CI ($<0.01, 0.20$), $F(1, 40) = 0.88$, $p = .35$. Heavier individuals showed a higher breathing rate after handling. However, catching latency was not influenced by body mass, $R^2 = 0.04$, bootstrap 95% CI ($<0.01, 0.18$), $F(1, 40) = 1.06$, $p = .31$, or body condition, $R^2 = 0.09$, bootstrap 95% CI ($<0.01, 0.26$), $F(1, 40) = 3.40$, $p = .07$. We then computed the variables “breathing rate adjusted for catching latency” and “breathing rate adjusted for body mass” by calculating the residuals from the regressions of breathing rate on catching latency and breathing rate on body mass. None of the four personality traits was significantly related to breathing rate, breathing rate adjusted for mass or for catching latency when considering the two trials separately (see Table 1). In addition, none of the four personality traits was significantly related to breathing rate adjusted for mass averaged across the two trials: activity, $r(39) = -0.12$, 95% CI ($-0.41, 0.19$), $p = .45$; reaction to startle, $r(39) = 0.06$, 95% CI ($-0.25, 0.36$), $p = .72$; exploration, $r(39) = -0.09$, 95% CI ($-0.39, 0.22$), $p = .56$; neophobia, $r(39) = -0.16$, 95% CI ($-0.45, 0.15$), $p = .31$ (Figure 1).

Discussion

This study investigated whether breathing rate during handling can be a reliable predictor of personality in zebra finches. Response to handling stress in the form of breathing rate was found to be consistent within individuals. However, our results indicate that individual variation in response to handling stress is not associated to individual variation in personality in female zebra finches, as least as assessed from the methodology used in David et al. (2011), thus, casting doubts on the extent to which response to handling stress should be used as a proxy to assess personality.

In particular, we found no evidence for a relationship between exploratory tendency or neophobia and breathing rate, contrary to

what has been previously reported in great tits (Carere & van Oers, 2004; Fucikova et al., 2009). The same was true of two other different personality traits, activity and reaction to startle. In addition, contrary to what was observed in great tits (Carere & van Oers, 2004), we found that breathing rate was positively correlated with body mass in female zebra finches, such that heavier individuals had a higher breathing rate during handling. This might be explained by the tight relationship between metabolism and body mass (Clarke & Johnston, 1999; McNab, 2002) and by the fact that metabolic rate depends on oxygen consumption and is directly related to ventilation rate in passerines (Arens & Cooper, 2005). The absence of a relationship between personality and breathing rate is particularly interesting as some evidence suggests that basal metabolic rate is heritable in zebra finches (Rønning, Moe, & Bech, 2005). Indeed, personality traits are supposed to be independent from any motivational or life-history state (Réale et al., 2007), although few studies verified this assumption so far (Fucikova et al., 2009).

Several explanations, based on methodological issues or biological differences between species, can however be advanced to explain the observed discrepancy between our results and those obtained in great tits (Carere & van Oers, 2004; Fucikova et al., 2009). From a methodological point of view, this study only used females as study subjects. The possibility then remains that breathing rate is related to male personality but not to female one, although this seems unlikely. In addition, Carere and van Oers (2004) estimated breathing rate in great tits during a 60-s period, which is substantially longer than in this study. Nonetheless, we believe that this discrepancy cannot be responsible for the results we obtained, as zebra finches get stressed from both human presence and catching. Breathing rate is, thus, likely to reflect a stable stress state in our experiment rather than the escalation of stress. Finally, Carere and van Oers (2004) assessed individual personality only from reaction of birds toward a novel object. Because

Table 1
Relationships Between Each Personality Trait and Breathing Rate, Breathing Rate Adjusted for Mass, and for Catching Latency

| Variable | Exploration | | Activity | | Neophobia | | Reaction to startle | |
|--|---------------|----------|---------------|----------|---------------|----------|---------------------|----------|
| | <i>r</i> | <i>p</i> | <i>r</i> | <i>p</i> | <i>r</i> | <i>p</i> | <i>r</i> | <i>p</i> |
| | (95% CI) | | (95% CI) | | (95% CI) | | (95% CI) | |
| Breathing rate | | | | | | | | |
| 1st trial | -0.17 | 0.29 | -0.11 | 0.51 | -0.16 | 0.31 | 0.17 | 0.28 |
| | (-0.45, 0.15) | | (-0.40, 0.21) | | (-0.45, 0.15) | | (-0.14, 0.46) | |
| 2nd trial | -0.11 | 0.49 | -0.06 | 0.70 | -0.16 | 0.31 | 0.10 | 0.54 |
| | (-0.40, 0.20) | | (-0.36, 0.25) | | (-0.45, 0.15) | | (-0.22, 0.39) | |
| Breathing rate adjusted for mass | | | | | | | | |
| 1st trial | -0.13 | 0.42 | -0.13 | 0.43 | -0.15 | 0.34 | 0.12 | 0.47 |
| | (-0.42, 0.18) | | (-0.42, 0.19) | | (-0.44, 0.16) | | (-0.20, 0.41) | |
| 2nd trial | -0.04 | 0.80 | -0.10 | 0.54 | -0.15 | 0.35 | <0.01 | 0.96 |
| | (-0.34, 0.27) | | (-0.39, 0.22) | | (-0.44, 0.17) | | (-0.31, 0.30) | |
| Breathing rate adjusted for catching latency | | | | | | | | |
| 1st trial | -0.15 | 0.36 | 0.02 | 0.90 | -0.02 | 0.89 | 0.08 | 0.64 |
| | (-0.44, 0.17) | | (-0.29, 0.33) | | (-0.32, 0.29) | | (-0.24, 0.38) | |
| 2nd trial | -0.11 | 0.49 | -0.06 | 0.70 | -0.16 | 0.31 | 0.10 | 0.54 |
| | (-0.40, 0.20) | | (-0.36, 0.25) | | (-0.45, 0.15) | | (-0.22, 0.39) | |

Note. CI = confidence interval. Breathing rate adjusted for mass and adjusted for catching latency was computed as the residuals of the linear regression of breathing rate on respectively body mass and catching latency. Degrees of freedom equal 40 for each test.

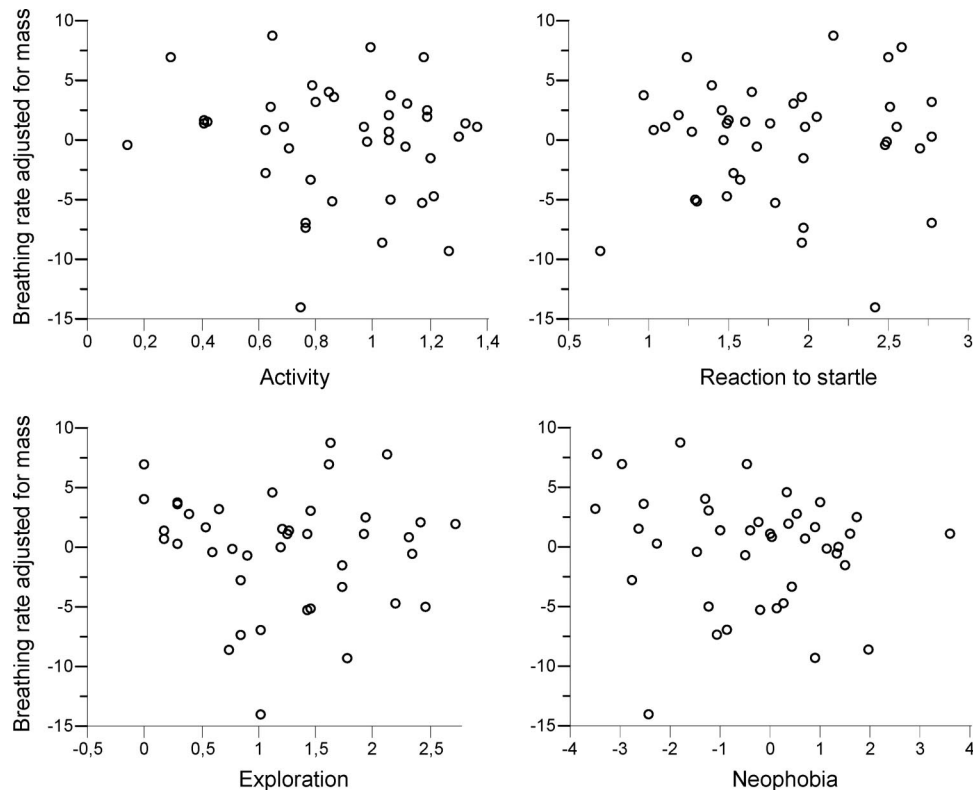


Figure 1. Nonsignificant relationship between each personality traits and breathing rate (averaged across the two trials) adjusted for mass during handling. Breathing rate adjusted for mass was computed as the residuals of the linear regression of breathing rate on body mass.

animal personality is most probably composed of several dimensions, it might be difficult in practice to assess it from one single trait, and inference of this sort should then be performed with extreme caution in future studies. For instance, in zebra finches, social dominance within groups is influenced by a set of personality traits such as exploration or activity (David et al., 2011). Conversely, obstinacy, which is not related to other personality traits but shows high repeatability, has no effect on social dominance in this species (David et al., 2011).

Another possibility is that the relationship between personality and physiological traits may vary between species. Great tits are obligate cavity nesters, usually nesting in a hole in a tree, whereas zebra finches are not (Zann, 1996; see however Griffith, Pryke, & Mariette, 2008). Some evidence suggests that chronic exposition to CO₂ during development may affect the development of ventilatory behavior in cavity-nesting bird species (Williams & Kilgore, 1992), eventually affecting the observed relationship between personality and breathing rate in adult birds. Whatever the causes of observed discrepancies between studies, additional work is clearly needed to assess the extent to which breathing rate can be used as a reliable proxy for overall personality in great tits and other species.

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