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Chapter 4

Handling stress as a measurement of personality in great tit nestlings (*Parus major*)

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Abstract

Interest in personality is growing in a wide range of disciplines, but only in a few systems it is possible to assess the survival value of personality. Field studies looking at the relationship between personality and survival value early in life are greatly hampered by the fact, that personality can at present only be assessed after individuals become independent from their parents. In passerines, for example, this is often after a period of intensive selection for survival on fledglings. The main aim of this study is therefore to develop a method to measure personality before this period of selection. For this purpose we developed the handling stress test (HS test). We measured HS in 14 days old great tit nestlings by counting the number of breast movements (breath rate) in four subsequent 15-second bouts for one minute; before and after they were socially isolated from their siblings for 15 minutes. To calculate the repeatability of HS we repeated the test six months later. To assess the relationship between HS and exploratory behaviour, we correlated the outcome of both tests. We ran tests both on birds of lines selected for extreme personality and on wild birds from a natural population. We found that birds selected for fast exploration reacted more to HS compared to birds selected for slow exploration and that HS was repeatable in different life phases. We confirmed this by finding an increase in HS with increasing exploratory scores in wild birds. These results show that we can use the HS test as a measurement of personality, making it a potential tool for studying the relationship between personality and survival value early in life.

Introduction

Interest in animal personality is growing in a wide range of disciplines. Personality affects consistent behaviour in different situations and is also referred to as temperament, coping style, coping strategy or behavioural syndrome in several research fields (Koolhaas *et al.* 1999; Sih *et al.* 2004a; Reale *et al.* 2007). Variation in personality has been studied in a number of animal species (Gosling 2001). Personality traits are shown to be heritable (van Oers *et al.* 2005a; Fidler *et al.* 2007), genetically correlated (Drent *et al.* 2003; van Oers *et al.* 2004a) and to have limited plasticity (van Oers *et al.* 2005b). Although individuals consistently differ in their behavioural responses towards the same stressor, their response can slightly but not rapidly change over time by gaining experience and in dependence on the changing context (Groothuis & Carere 2005). Studies on fitness consequences have shown that personality traits are under natural selection (Dingemanse & Réale 2005; Smith & Blumstein 2008; Van Oers *et al.* 2008). One of the most critical moments of selection is in the first weeks after juveniles achieve independence of their parents (Drent 1984; Hall *et al.* 2001). Nevertheless, there are few studies combining lifetime fitness of individuals with their personality measured in juvenile age in natural populations (Smith & Blumstein 2008). The main reason for this is that juvenile personality is often unknown (Boon *et al.* 2007). Such studies could potentially be carried out on e.g. fish, which are independent from hatching (Wilson *et al.* 1993), on juveniles bred in the lab and then released (Bremner-Harrison *et al.* 2004) or by using the personality of the parents as a measurement of the personality of their individual offspring. Using parent personality scores can be misleading, since the personality of an individual offspring is expected to be different from the mid-parent value. Early developmental effects (Carere *et al.* 2005; Arnold *et al.* 2007) and non-additive genetic effects (van Oers *et al.* 2004c) can generate variation between individuals within broods and consequently changes in frequencies of personality types within population. Hence, it is crucial to have a personality measurement for each individual before juveniles become independent and selection takes place.

In this study we therefore aim to develop such a measurement, by using our model system the great tit. In this passerine species, we can recognize two extremes at the ends of continuous behavioural axes. On one extreme, so called fast explorers, explore a novel environment quicker and more superficially compared to slow explorers (Verbeek *et al.* 1994). Fast explorers are bolder in nest defence (Hollander *et al.* 2008) and new situations (Verbeek *et al.* 1994), more aggressive towards others and more often initiate contests (Verbeek *et al.* 1996). They are more routine in habits, copy feeding behaviour of slow explorers and do not develop new food searching strategies (Marchetti & Drent 2000). In contrast, slow explorers are shyer, initiate fewer contests and are less routinous but more flexible than fast explorers (see review Groothuis & Carere (2005)).

In our study we measure handling stress during an interval, when birds are separated from their siblings and parents. Hence, handling stress here is a change of breath rate during four 15-s breath rate intervals within a 1-min period of handling, 15-min period of isolation and again 1-min period during the Handling stress test. Handling stress

indicates the stress caused by handling and by the period of isolation. In fish are handling stress and opercular beat rate used as a measure of metabolic rate and/or stress response (Brown *et al.* 2005; Bell *et al.* 2010). Handling stress and early separation are routinely used as stressors in developmental and physiological studies of mammals and birds (Mills *et al.* 1993; Beane *et al.* 2002). Mills *et al.* (1993), for example, isolated Japanese Quails selected for high (HSR) and low sociality (LSR) and studied their behaviour during isolation. Isolated HSR quails were more active than LSR ones in the isolation, but if they were with the others, no differences were visible. Kitaysky *et al.* (1999) have shown that it is possible to measure handling stress also in juvenile birds. They used handling and restraint induced stress to measure increased corticosterone levels in juvenile kittiwakes (*Rissa tridactyla*). A similar test showing varying responses in different personality types has been performed on full grown independent great tits. Carere and van Oers (2004) used a handling stress protocol to show a relationship between breath rate and boldness in six month old birds from lines selected for early exploratory behaviour. They found that shy individuals had higher breath rates compared to bold ones, but there were not significant differences in performance before and after isolation. On account of these results we developed a similar test for nestlings to measure variation in handling stress. We tested selection line and wild nestlings in a similar way as Carere and van Oers (2004), but to study the increase of the breath rate during handling, we divided each minute into 4 equal bouts of 15 seconds. In great tits the corticosterone level culminates 10 minutes after handling (Cockrem & Silverin 2002). Therefore, and for practical reasons, we used a 15 minute interval of social isolation. For comparison with the study of Carere and van Oers (2004) and to study repeatability of handling stress in different life phases, selection line birds were also tested for handling stress at an age of approximately six months. In order to create a possibility to compare studies that used exploration as expression of personality we correlated handling stress test (age: 14 days) and novel environment test in wild birds.

Methods

Study subject and area

The great tit is a common non-migratory passerine that lays clutches of 5 – 12 eggs that hatch after about 13 days of incubation. Nestlings are full grown on day 13 and fledge between 16 and 23 days after hatching. Fledglings are still fed by their parents for 2 to 3 weeks. There is high mortality in the first days after fledging (two to three weeks when parents still guide fledglings) and the period right after the fledglings become independent (Drent 1984; Naef-Daenzer *et al.* 2001). Mortality rates can reach up to 10 % in the first days after fledging, and predation is expected to be the main cause of this (Naef-Daenzer *et al.* 2001); while mortality after reaching independence (30-50%) is caused by starvation and due to a lack of experience in food searching (Drent 1984).

Data on selection line birds were collected in 2007. We used birds that originated from the fourth generation of a bidirectional artificial selection experiment (Drent *et al.*

2003). Selection was done for fast and slow early exploratory behaviour (EEB), which is a combination of the outcome of a novel environment and a novel object test, resulting in lines of fast and slow exploring birds. EEB is genetically correlated with other personality traits (van Oers *et al.* 2005a). The eggs of captive pairs originating from the selection lines were collected and cross-fostered to wild foster parents. Nestlings were brought to the lab on day 10 after hatching and hand reared until independence. Subsequently, birds were housed individually in home cages of 0.9x0.4x0.5 m, with a solid bottom and top, side and rear wall, a wire-mesh front and three perches. Nestlings and fledglings were provided with a beef hart mixture, supplemented with insect food and water (see Drent *et al.* (2003)).

Data on wild great tits were collected from a nest box population in the areas of Westerheide and Warnsborn near Arnhem, the Netherlands (5°50'E, 52°00'N) in 2002, 2004 and 2006. This area with a mixed tree species composition has 240 nest boxes regularly distributed. This population is also the one from which selection line birds originated. From April onwards we checked nest boxes twice a week until females were incubating full clutches. To know the exact date of hatching we visited these boxes each day from the expected hatching date onwards. Nestlings were banded with an individual metal ring on day 10. After the fledglings reached independence from parents, we started an intensive capture-mark-recapture program lasting until the next breeding season. In this program we carried out mist-netting twice a week and roosting control of nest boxes in November and March. If birds were caught for the first time we measured tarsus, weight, gave them an individual metal band and took a blood sample. To test their personality, we brought them to the laboratory (described below).

Handling stress protocol of 14 day-old nestlings

We conducted handling stress (HS) tests on selection line and wild nestlings on day 14 after hatching between 0900 and 1600 hours. We started the procedure by taking all nestlings from the nest box and putting them in a cotton bag. We closed the nest box entrance to stop parents from entering the box. In a shady location we took a nestling from the bag and held it in the hand loosely on its back. We thereby fixed it by keeping its head between thumb and index finger (Fig.1).

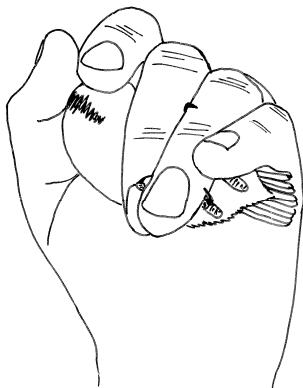


Figure 1. Grip when counting breath rate in the HS test.

We held the nestling loosely on its back in the hand, fixing it by keeping its head between thumb and index finger and gently laying the middle finger on the breast.

We counted the number of breast movements (breath rate) during a one minute handling period. The handling period was divided into four 15-second bouts (i.e. obtaining four data points per one minute). Thereby, HS is the increase in breath rate over a one-minute handling period. After this we put each individual into a wooden box for 15 minutes. The wooden box (32 x 12.5 x 6 cm) was divided into 12 compartments of equal size (6 x 6 x 6 cm) partitioned by 5 mm thick plywood. The box was covered with a sliding lid with holes. Nestlings were thereby spatially and visually but not auditory isolated from their siblings (social isolation). After we put the nestling in a compartment, the next nestling was taken from the cotton bag, tested in the same way and put into the next compartment. This was repeated until we tested the whole brood. After the first nestling had been in the wooden box for 15 minutes, we took it from the box and measured breath rate in the same way as described above. Thereby, Period 1 and 2 refer to the periods of one minute before and after social isolation subsequently. After a nestling was tested, it was put back in the cotton bag. When all birds of a brood were tested, we weighed them with a pesola to the nearest 0.1g, measured their tarsus with sliding callipers to the nearest 0.1 mm, and placed them back in the nest box. Because temperature in the test box can affect body temperature and thus breath rate, we measured air temperature in the shadow at the location of the test. We thereby assume that temperature outside is correlated with temperature inside the test box. The HS protocol did not differ between nestlings from the selection lines in the laboratory and wild nestlings.

The age of 14 days is the most suitable age for carrying out the HS test. Nestlings are fully feathered and have a well developed thermoregulation. In a study on the physiological reaction of great tit nestlings to vocal information, Ryden (1980) showed that 10-day old nestlings reacted to alarm calls differently than to other calls, indicating that the stress response already developed at this age. This difference, however, was much greater in a similar experiment on 14-day old nestlings (Ryden 1982), which shows that the response gradually develops. In addition, an earlier pilot study has shown that 10-day old nestlings are less responsive to handling stress than 14-day old nestlings (unpublished data). If we would conduct the HS on older nestlings, the risk of premature fledging would be too high.

Handling stress protocol of full grown birds from selection lines

To test the repeatability and consistency of the HS test in different life phases, we repeated the test with the selection line birds when they were full grown. This repetition of the HS test might include habituation. Since Dingemanse et al. (2002) found that habituation was resolved in exploratory behaviour after 3-5 months; we decided to test our full grown birds at an age of approximately 6 months.

Since it is impossible to catch and handle full grown birds in the same way as nestlings, we used a modified form of the method developed by Carere and van Oers (2004). After we caught a bird from its individual cage (which is a stressful action for a bird), the bird was held in the hand. We counted the breath rate during a one minute-long

handling period, just as we did for the nestlings. After this we put each individual into a cotton bag for five minutes. Subsequently we measured breath rate again in the same way as described above.

Novel environment test

Exploratory behaviour was measured using the novel environment test developed by Verbeek et al. (1994). Individuals were tested alone in a sealed room (4.0×2.4×2.3 m) with five artificial trees. Birds were introduced into the room without handling by darkening the cage with a curtain, opening the sliding door and turning on the light in the test room. We used the total number of flights (movements between trees) and hops (movements within trees) within the first 2 min as a measure of exploratory behaviour. Faster explorers have higher scores compared to slower explorers by definition. We corrected data for date of capture based on the finding that behaviour changes with capture date within individuals (see Dingemanse et al. (2002)). After all birds had been tested and weighed, they were released at the place of capture.

Ethical note

Selection line birds were reared by foster parents in the wild. At an age of 10 days after hatching they were transported to the laboratory and hand-reared until independence. Earlier research has shown that there are no negative effects on growth and condition of the nestlings. Survival in the laboratory is about 96%. Wild birds were kept caught in laboratory for a period of 14-24 hours before conducting the novel environment test. After this test the birds were released at the place of catching. Pilot research has shown that there was no effect on the social status and no increase of mortality and dispersal by this short period of absence from the field. Permission for great tit collection, hand rearing, behavioural tests and blood sampling on wild birds was granted by the legal committee “KNAW Dier Experimenten Commissie (DEC-KNAW) license nr. CTO.03-02 and CTO.03-05 to KvO and PD and CTO.07-05 and CTO.07-06 to EF, KvO and PD.

Statistical analyses

Handling Stress

We used a generalized linear mixed-effects model approach (GLMM), with the number of breaths per 15 second-bouts (in total eight per individual: four bouts in Period 1 and Period 2) as the dependent variable to describe the HS test. To be able to calculate the slope of breath rates in each Period, we included HS (four 15-second bouts) nested within Period (fixed effect) as a continuous variable in a GLMM (*lmer* in R package *lme4*). We also included Period (fixed effect) and ‘individual’ (random effect) in the model. We included weight and tarsus length, date of testing, time of day, temperature and temperature squared as covariates. Weight and tarsus length represent condition of the bird. Weight correlates with the survival in tits (Tinbergen & Boerlijst 1990; Both *et al.* 1999) and tarsus length is a linear measure of body size (Garnett 1981). Date of testing represents the hatching date

which correlates with the caterpillar peak important for the nestling condition (Naef-Daenzer & Keller 1999). Because temperature in the test box can affect body temperature and thus breath rate, we measured air temperature in the shadow at the location of the test and temperature squared (because of an expected non-linear relationship between breath rate and temperature).

To investigate the relationship between HS and exploratory behaviour we used the above described model to derive individual estimates for HS. For calculating these estimates for each individual separately and each Period of HS separately we included the interaction between individual and HS (nested in Period) into the model. The obtained estimates are therefore a measurement of the individual deviations from the mean HS (i.e. individual deviations from the slopes). All models for each subgroup (selection lines, wild, nestling or full grown) were specified in an identical way (including the same covariates), allowing comparison of the estimates. To investigate whether the selection lines differed in their response to handling, we used t-tests where equal variances were assumed.

We used 236 wild individuals from 112 nests (mean = 2.72 individuals per nest) that were tested for the HS as nestlings and tested for exploratory behaviour as full grown birds, to look at the relationship between HS and exploratory behaviour. To account for the possible effect of pseudo-replication in this dataset, we ran a linear mixed-effect model with exploratory score as the dependent variable and the HS estimate as the independent, thereby including brood as a random effect.

Repeatability

We calculated the repeatability of HS for 37 birds from the selection lines by using the individual estimates derived from the GLMM's at the age of 14 days and the estimates derived from the model at the age of 6 months. We used the estimates of HS as the dependent variable and individual as a factor in an ANOVA. The repeatability, the proportion of the phenotypic variance explained by the individual (Falconer & Mackay 1996), was calculated following Lessells and Boag (1987) and its standard errors following Becker (1984).

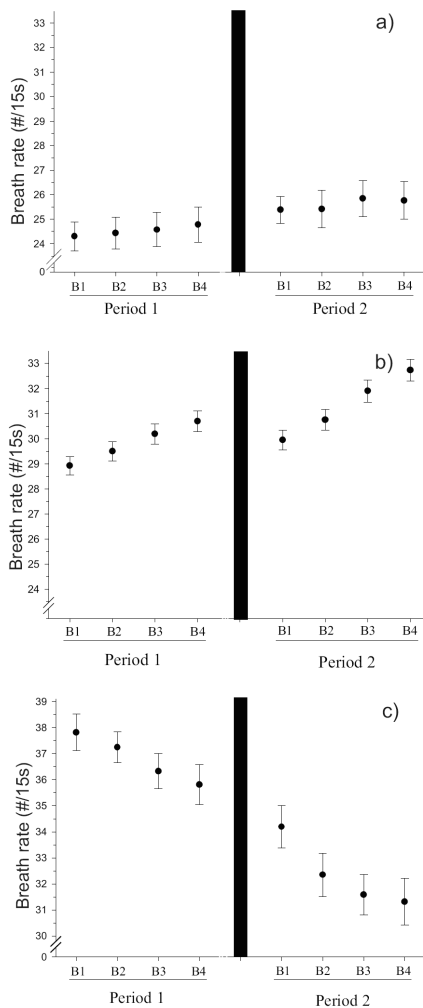
We used the software packages R 2.6.1 (R Development Core Team 2007), and SPSS 15.0 (SPSS INC., Chicago, IL) for all statistical analyses. All statistical tests were two-tailed.

Results

The 15 minute-interval was mostly long enough to test the whole nest. However, this interval sometimes increased up to 25 minutes in large broods (10-12 nestlings). We used the first 15-second bout in Period 2 as a measure of the effect of isolation to compare the breath rate in small broods (up to 10) and larger broods. We found that our results were not affected by the longer isolation (GLMM; brood size, $\chi^2_1=10.31$, $p=0.47$). The sequence in which nestlings were taken from the nest box did not affect their breath rate (GLM; Bout 1

in Period 1, $\chi^2_1=0.012$, $p=0.91$). We also did not find any observer effect (3 observers) on breath rate (GLMM; All bouts, $\chi^2_1=0.015$, $p=0.9$).

In 14 days-old nestlings from selection lines and wild birds respectively, breath rate during handling is increasing (Fig. 2a and Fig. 2b) both before (Period 1) and after isolation (Period 2). In selection line birds there is no difference between the slopes of this increase in Period 1 compared to Period 2 (GLMM; Period*HS, $F_{1,256}=0.897$, $p=0.41$), although the general breath rate level is higher in Period 2 (GLMM; Period, $F_{1,257}=16.36$, $p<0.001$). Wild birds react stronger to handling in Period 2 compared to Period 1 (GLMM;



Period*HS, $F_{1,1950}=7.177$, $p=0.007$; Fig. 2b). In full grown birds from the selection lines, HS is characterized by a significant decrease in the number of breaths per 15-second bout (Fig. 2c; GLMM; HS, $F_{1,256}=27.61$, $p<0.001$). Thereby breath rate is lower in Period 2 compared to Period 1 (GLMM; Period, $F_{1,256}=20.31$, $p<0.001$). There was no difference in HS between the two Periods (GLMM; Period*HS, $F_{1,256}=0.616$, $p=0.43$).

Individuals differ from each other in HS and this is true for nestlings and full grown birds from the selection lines and for wild nestlings (Table 1). Nestlings from the fast selection line showed a higher response in Period 2 compared to slow explorers ($t_{2,35}=2.809$, $p=0.008$; Fig. 3a), but did not differ from each other in Period 1 ($t_{2,35}=1.438$, $p=0.159$; Fig. 3a). This was confirmed in the measure taken on the same birds at the age of six months; birds from the fast selection line reacted more strongly to handling stress than birds from the slow line in Period 2 ($t_{2,35}=2.140$, $p=0.039$), but they did not differ from each other in Period 1 ($t_{2,35}=-1.210$, $p=0.234$).

Figure 2. Breath rate (number of breast movements per 15-second bout) with standard error of 14 days-old nestlings from (a) birds from lines selected for fast and slow exploratory behaviour and of (b) wild population. Fig. 2(c) shows the results for the same birds from the selection lines at the age of six months. For (a) and (b), measurements were taken one minute (4 bout intervals of 15 sec, B1, B2, B3, B4) before a 15 minute of social isolation (Period 1) and one minute after isolation (Period 2). For figure (c), measurements were taken one minute directly after capture from their home cage (Period 1) and after 5 minutes of isolation in a catching bag (Period 2).

Table 1. Results of a GLMM of breath rate[‡] within two handling periods (before and after social isolation) with normal errors and type III sums of squares. The dependent variable is the breath rate per 15-second bout. Individual is included as a random effect. Fixed effects are HS[§] nested within Period[¶], Period and the interaction between individual and HS nested within Period[¶]. Control variables are April day, Temperature, Temperature², Time, Tarsus, and Weight. F and p values given are for the inclusion of the variable in the final model without non-significant terms. The test was done in wild great tit nestlings at an age of 14 days and nestlings from selection lines at an age of 14 days and of 6 months.

Variable	Wild (14 d, n=236)		birds		Selection (14 d, n=37)		lines		Selection (6 m, n=37)		lines	
	Df	F	P	Df	F	P	Df	F	df	F	P	
HS(Individual*Period) [‡]	471	4.048	0.000	73	4.522	0.000	73	5.898	0.000	73	5.898	0.000
HS (Period)	1	262.923	0.000	1	3.419	0.660	1	66.086	0.000	1	66.086	0.000
Period	1	3.389	0.066	1	5.328	0.022	1	48.682	0.000	1	48.682	0.000
April day	1	2.938	0.088	1	NA	NA	0	NA	NA	0	NA	NA
Temperature	1	0.063	0.802	1	0.230	0.635	0	NA	NA	0	NA	NA
Temperature ²	1	0.036	0.851	1	0.207	0.652	1	0.009	0.927	1	0.009	0.927
Time	1	3.253	0.073	1	0.804	0.377	1	0.286	0.597	1	0.286	0.597
Tarsus	1	9.385	0.002	1	1.399	0.246	1	0.511	0.480	1	0.511	0.480
Weight	1	2.628	0.106	1	0.372	0.547	1	0.006	0.939	1	0.006	0.939

Breath rate[‡] number of breast movements during a 15-second bout

HS[§] change of breath rate during four 15-sec breath rate intervals within a one-min Period (slope); indicates the stress caused by handling

Period (of handling)[¶] period before (Period 1) and after (Period 2) isolating chicks from their siblings for 15 minutes; indicates difference between the HS in Period 1 and 2 within one individual

HS(Individual*Period)[‡] interaction between individual and isolation stress, where HS is nested within Period; indicates whether individuals differ in their slope for handling (stress caused by handling) between the two Periods

When comparing the outcome of the HS test on wild nestlings with their exploratory score, we found a relationship between exploration and handling stress. To visualize this we have plotted the raw breath rate values for individuals categorized into four personality types according to their personality score (Fig. 3b). We found that, like in the selection line birds, individuals with high exploration scores (fast exploring birds) showed a stronger response to handling than individuals with low exploration scores (slow exploring birds). The effect, however, was significant only in Period 1 ($\beta = -0.68$, $n=233.7$, $p=0.024$), but not in Period 2 ($\beta = -0.18$, $df=233.4$, $p=0.556$). To measure the repeatability of HS we used the individual estimates for HS in Period 2 derived from the above mentioned GLMM's for 37 birds at an age of 14 days and six months later. We thereby found a repeatability of 0.338 ± 0.147 .

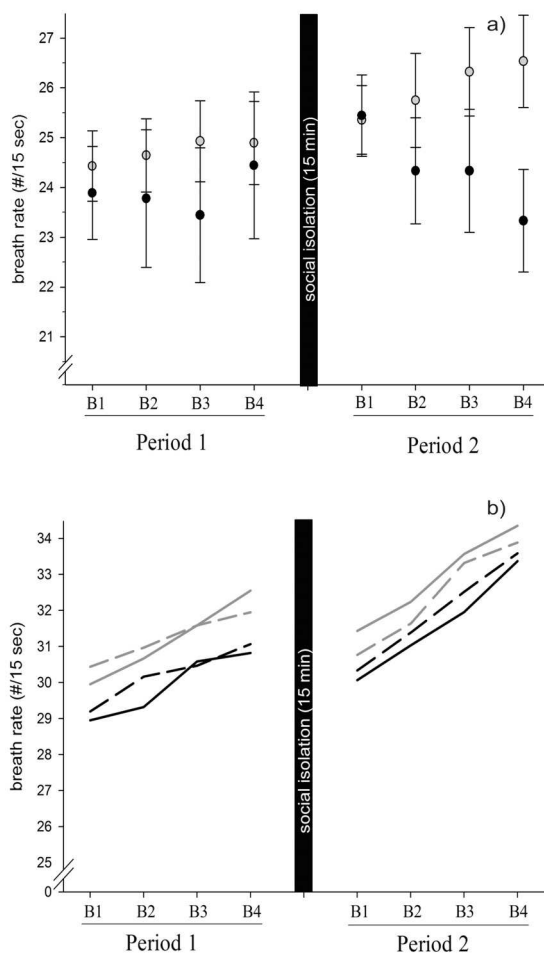


Figure 3. Mean breath rates (number of breast movements per 15 seconds) of 14 day-old birds during the two handling periods (see text for details) from (a) lines selected for slow (solid black symbols \pm SE) and fast (solid grey symbols \pm SE) exploratory behaviour, and (b) a wild population, separated into the 25% with lowest exploratory scores (very slow - black solid line), the 25th-50th percentile (slow - black dashed line), the 51st-75th percentile (fast - grey dashed line) and the 25% highest exploratory scores (very fast - grey solid line). All values represent raw data.

Discussion

The aim of this research was to develop a simple personality test on nestlings in the field. We found that handling increases the breath rate in selection line as well as in wild nestlings, and that this HS was different after a 15 minute period of social isolation. Our study shows that HS is repeatable in different life phases and that HS is associated with another personality trait, exploratory behaviour, an accepted proxy for personality in great tits.

Wild nestlings show a relationship between HS and exploratory behaviour in Period 1, while in selection line nestlings this relationship appears in Period 2. We offer three hypotheses that could explain these differences. First, the difference between wild and selection line birds might reflect the difference in the interaction between genes (biological parents) and the rearing conditions (rearing parents). Different gene-environment interactions during breeding may have resulted in a change in physiological pathways in time. As a consequence of an increase in stress level (Svensson *et al.* 2001; Nyberg *et al.* 2004) they will activate different physiological pathways in time or with the level of stress. The difference in response between Periods then reflects a difference in time between the levels of stress (HS and HS + isolation). To provide evidence for this hypothesis a cross-fostering experiment is needed. Second, selection line nestlings have different experience with handling compared to wild nestlings. Wild nestlings grew up in their own parents' nest and had no experience with handling before the HS test. They could therefore have experienced the first handling as a moderate challenge and therefore showed differences in HS, whereas the isolation and Period 2 increased the HS to such high level that differences in behaviour disappeared (overreaction). On the contrary, selection line nestlings were hand reared since an age of 10 days. Because handling is a more common situation for them, the isolation may seem to be a stronger stressor than handling. Third, differences between selection line and wild nestlings could be caused by low statistical power. Small sample size in the selection line birds cause large standard errors, which could hide a possible effect in Period 1, where the variation is the lowest. To our opinion, the first and second hypotheses are the most likely ones to explain the difference between wild and selected individuals. The second hypothesis can be also taken as a special case of the first, since hand rearing can be understood as a part of the rearing conditions. In such a case it is interesting to ask whether the foster parents or hand rearing have a stronger effect on the reaction of juveniles towards stress.

The HS test in the nestling stage may seem as an unnatural stimulus, since these nestlings are in nest boxes and are normally not isolated from their siblings. However, the juveniles are fully grown and fledge 2-7 days after the experiment and are still completely dependent on their parents for another 10-20 days. In that period they meet predators and may become temporarily isolated from siblings and/or parents. Thus, the physiological response towards such isolation already develops in the last part of the nestling period. Our experiment simulates such a state and shows that juveniles indeed most likely react to isolation. Moreover, 14-day old nestlings have been shown to express a different stress

response towards conspecific alarm calls compared to a control alarm call (Ryden 1980), indicating that the physiological response towards threats is already developed in these old nestlings.

We found an opposite response in HS of birds at the age of 14 days compared to birds at the age of six months. Likely taking nestlings from a nest box does not act as a strong stressor, but catching of full grown birds from their home cage highly increases their stress. Thus, isolation might act as a stressor in nestlings, while keeping adult birds in a bag decreases their stress response (Carere & van Oers 2004). Alternatively, stress might increase breath rate in nestlings, but decrease the breath rate of adults.

There are two reasons why the HS test cannot be taken as a substitute for the novel environment test (the standard test of personality in many passerine populations). First, although we found a significant correlation between handling stress and the exploration score in the novel environment test, it explains only a small part of the variation. It is possible that apart from similar ones, we also measure different components of personality in the response to handling compared to the response to novel environment. Personality is multidimensional and the behaviour we observe is context dependent; responses differ with age, situation and increasing experience (van Oers *et al.* 2005b). This leaves large scope for future investigations on why individuals differ in HS and how this develops. Then we may find stronger correlations with other types of behaviour and discover consequences of the relationship with exploratory behaviour. Second, the HS test is not very practical for studying the relationship between personality and fitness in an adult population. When catching immigrants and full grown birds in mist nets, only the first individual can be taken directly from the net and tested for HS without being affected by uncontrollable factors like time since catching.

In conclusion, our results show that with the HS test we have a measure of personality of nestlings and can measure the variation between and within nests in the whole breeding population. This opens up possibilities to assess the effect of individual personality on mortality and dispersal in a critical period right after fledging. The HS test therefore will be useful when studying the behavioural composition of a local population of juveniles and for studying the ecology and evolution of personality.

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