Animal Behaviour 84 (2012) 279–287

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Commentary

A simple cage test captures intrinsic differences in aspects of personality across individuals in a passerine bird

E. Kluen [a,*], S. Kuhn [b], B. Kempenaers [b], J. E. Brommer [a]

[a] Bird Ecology Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland
[b] Department of Behavioural Ecology & Evolutionary Genetics, Max Planck Institute for Ornithology, Seewiesen, Germany

ARTICLE INFO

Article history:
Received 9 November 2011
Initial acceptance 6 January 2012
Final acceptance 5 April 2012
Available online 26 May 2012
MS. number: 11-00903R

Keywords:
animal personality
blue tit
cage
Cyanistes caeruleus
dopamine receptor
DRD4
escape behaviour
exploratory behaviour
novel object
repeatability

Personality tests are best carried out in an artificial, standardized environment. There is a need to develop reliable approaches for testing wild individuals in a manner that minimizes harm or stress to individuals while allowing maximal flexibility (e.g. a portable set-up or short protocols) for researchers. We developed a behavioural assay of personality traits for a wild-caught bird placed in a standard bird cage, which takes approximately 15 min per assay. After 10 min acclimation, we quantified neophobia-related behaviour (in terms of time spent in different parts of the cage), activity (movement through the cage by hops or short flights) and the time it took to escape from the cage after opening the cage door in 293 assays of 224 individual blue tits, *Cyanistes caeruleus*, carried out during three consecutive winters. Neophobia-related behaviour and escape time were significantly repeatable and showed no annual or within-year temporal variation or differences between sexes or age classes. Escape time was associated with one of two single nucleotide polymorphisms in exon 3 of the *DRD4* gene that we considered as candidates. This gene is the prime candidate for novelty-seeking behaviours and this genomic region has been found to associate with exploration score in the closely related great tit, *Parus major*. We conclude that our assay can capture repeatable and heritable differences in aspects of personality among wild individuals.

Personality can be described as consistent behavioural responses over time and context displayed by individuals exposed to (stressful) stimuli. This consistency could arise because of genetic differences between individuals. Research on human behaviour has revealed several candidate genes underlying human behavioural traits (e.g. dopamine receptor D4 gene (*DRD4*) and the serotonin transporter protein (*SERT*); reviewed in Savitz & Ramesar 2004). More recently, polymorphisms in the *DRD4* gene have been associated with novelty seeking in mammals bred in captivity (horses, *Equus caballus*: Momozawa et al. 2005; monkeys, *Cercopithecus aethiops*: Bailey et al. 2007; dogs, *Canis familiaris*: Hejjas et al. 2007). In selection lines for fast and slow exploratory behaviour

* Correspondence: E. Kluen, Bird Ecology Unit, Department of Biosciences, PO Box 65, Viikinkaari 1, 00014 University of Helsinki, Helsinki, Finland.
E-mail address: edward.kluen@gmail.com (E. Kluen).

doi:10.1016/j.anbehav.2012.04.022

in great tits, *Parus major*, a single nucleotide polymorphism (SNP) in exon 3 of the *DRD4* gene (SNP830) is associated with exploratory behaviour (Fidler et al. 2007) and this association is also found in one of four wild great tit populations (Korsten et al. 2010).

In general, however, there are both genetic and nongenetic hypotheses to explain the maintenance of individual consistency in nature (e.g. Dall et al. 2004; Sih et al. 2004; Wolf et al. 2008). The empirical challenge is to understand the selective advantages or disadvantages of relatively inflexible differences in behaviour between individuals. To meet this challenge, we must first quantify personality under natural conditions in order to link it to individual performance. The quantification of personality under natural conditions is hindered by the fact that conditions surrounding the measurement are not under the researcher's control. Indeed, under natural conditions, it is possible that the individual itself has chosen, has been forced or just happens to be in a certain condition surrounding the measurement. Hence, the individual is interacting with the conditions during testing and therefore interpretation of the measurement is difficult. For example, the flight initiation distance (FID, e.g. Blumstein 2003) of an individual occupying a densely vegetated territory may be much shorter than for an individual in an open-habitat territory. Because the territory is fully confounded with the individual, repeated measures of an individual's FID within the same season are likely to be repeatable (cf. Dingemanse et al. 2010). However, in this example, behavioural differences are caused by the effect of the habitat in the territory and not by intrinsic differences between the individuals. Many other naturally varying conditions will, of course, have far less obvious impact on the recorded behaviours. For example, measurement of an individual's behaviour in the wild can be affected by conspecifics in its immediate surroundings (e.g. on a feeding table in winter owing to the presence of a dominance structure; Lambrechts & Dhondt 1986).

To help contrast individuals' behaviours, one can measure behaviour of individuals in an artificial, standard environment. This has been used to score exploration in great tits: individuals are caught in the wild, kept overnight, tested the following day in a 'novel environment room' and then returned to their place of capture (e.g. Dingemanse et al. 2002). A positive aspect of this approach is that the researcher can better control (environmental) variables that might affect the measured behaviour. For example, by keeping the animals captive for a longer period and feeding them ad libitum, one can control the possible effect of hunger on the measured behaviour. Negative aspects of the 'novel environment room' set-up are that it is labour intensive and often requires considerable resources to run it. In addition it requires that the animals need to be kept in captivity for a considerable period of time, which is not desired or possible in some situations (e.g. in the breeding season) or might be harmful in others (e.g. taking birds out of freezing temperatures and housing them inside (warmer) before releasing them (into cold), which causes physiological stress, Newton 1998). These factors make this testing approach relatively inflexible; it is difficult to test individuals in different ecological contexts, for example testing during the breeding season as opposed to the wintering season. An alternative approach is to employ a smaller, portable cage and keep an individual only for a short time, which would allow rapid testing of individuals in the field in varying contexts while minimizing the time an individual is in captivity. For example, Nilsson et al. (2010) used a bird cage to quantify the response to a novel object in migratory and nonmigratory wild blue tits, *Cyanistes caeruleus*. However, whether you can measure intrinsic aspects of behaviour of a wild individual with this technique still remains unknown.

Here, we describe a simple cage-based technique employed in blue tits. We quantified three behavioural traits: (1) neophobia-related behaviour, (2) activity (in terms of movement through the cage during a fixed time period) and (3) time to escape from the cage. To assess whether the behaviours we quantified were properties of an individual, we employed a two-step approach. First, we calculated the repeatability of these traits. Second, we tested for a genetic basis underlying the repeatable behaviours. Because we lacked sufficient information on relatives to calculate heritability, we tested whether the significantly repeatable behaviours were associated with variation in a particular genomic region, a polymorphism in exon 3 of the *DRD4* gene, which, as discussed above, has been shown to be associated with personality in the closely related great tit (Fidler et al. 2007; Korsten et al. 2010).

METHODS

The study was conducted in three consecutive winters (2007–2008, 2008–2009 and 2009–2010), on a population of blue tits living in a mixed boreal forest in southwest Finland (Tammisaari; 60°01′N, 23°31′E). In the centre of the study area we established a feeding station with three feeders (peanuts, sunflower seeds and fat balls). In the winter 2007–2008, birds were trapped between November and March and in the other winters between January and March. Birds were captured using a mist net, as they approached the feeders. All birds trapped were measured (tarsus and weight), ringed and sex and age were determined based on plumage characteristics (Svensson 1992). If a trapped bird was already ringed (locally breeding birds or previously trapped birds), a blood sample (ca. 60 μl) was taken from the brachial vein by venipuncture and subsequently the bird was subjected to behavioural testing in a cage. Blood sampling was done only once per individual per season (first time caught); if birds were retrapped within a season no blood sample was taken.

Testing of Behavioural Traits

Each bird was tested individually in a standard commercial bird cage (FOP Chiara, 59.5 × 36 cm and 60 cm high). The cage had three perches positioned at the bottom, middle and uppermost levels and had three doors (one on each side and one in the front of the cage). The cage was modified such that a plywood board was attached to the back of the cage (the side without an entrance) to allow the researchers to approach the cage without being seen. In addition, on the positions where feeders (four in total) could be attached to the cage, square pieces of plywood were attached to block these. Cages were positioned underneath a transparent shelter roof, outside. All cages were positioned in a line (at approximately 2 m intervals) facing the same direction (southwest; towards an open field), separated by wooden boards on two sides of the cage, preventing the birds from seeing each other.

Immediately after being measured, birds were released into the cage via a side entrance and left undisturbed there for 10 min. From personal observations, we noted that birds required some minutes after release in the cage to settle down, that is, to stop making alarm calls and rapid flights through the cage. After this habituation period, the researcher briefly entered his hand through the front door of the cage (as if hanging something from the roof of the cage). Immediately thereafter, filming of the bird commenced with a small, compact digital camera (Pentax Optio A20) positioned on a tripod placed ca. 50 cm from one of the sides of the cage. After 2 min, a novel object (a pink plastic toy pig measuring 6 × 5 × 8 cm), to which a hook (ca. 6 cm) made of thin metal wire was attached, was hung from the roof of the cage. We chose a pink-coloured novel object, following Verbeek et al. (1994) and Nilsson et al. (2010) who used a toy pink panther (see also Herborn et al. 2010; pink frog). The colour pink is not often encountered in