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Neophobia is linked to behavioural and haematological indicators of stress in captive roe deer

Chloé Monestier [a, *], Nicolas Morellet [a], Hélène Verheyden [a], Jean-Michel Gaillard [b], Eric Bideau [a], Anaïs Denailhac [a], Bruno Lourtet [a], Nicolas Cebe [a], Denis Picot [a], Jean-Luc Rames [a], A. J. Mark Hewison [a]

[a] CEFS, Université de Toulouse, INRA, Castanet Tolosan, France
[b] LBBE, Université Lyon 1, UMR CNRS 5558, Villeurbanne Cedex, France

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Neophobia is an important personality trait that allows animals to minimize exposure to threat. We investigated the existence of consistent individual differences in the level of neophobia in captive roe deer, *Capreolus capreolus*, using an experimental set-up. Our main objective was to explore the link between an individual's level of neophobia with behavioural and physiological responses measured during a stressful situation, i.e. capture and restraint, to facilitate characterization of neophobia in the wild. We found that the probability of initiating a feeding bout and the feeding efficiency over bouts both decreased in the presence of a novel object. However, there was pronounced variation in the degree to which individuals were affected by the experimental treatment. First, feeding efficiency decreased the most among individuals that reacted less markedly to an acutely stressful situation (capture). Second, latency between the first visit and the first feeding bout increased the most among individuals that had a higher concentration of fructosamine in their blood, an indicator of chronic stress. Our results indicate that individuals that are more neophobic (high latency to first feeding bout and low feeding efficiency in the presence of a novel object) are also less proactive (low behavioural response to capture, high levels of fructosamine), suggesting the existence of a behavioural syndrome. We conclude that behavioural and physiological parameters measured during capture provide reliable indicators of neophobia for roe deer, providing an exciting new avenue for the study of animal personality in the wild.

Differences between individuals in behaviour are often reported to occur within a population, both in the wild and in captivity (Gosling, 2001; Sih, Bell, & Johnson, 2004). Research in the field of animal personality (i.e. individual behavioural differences which are consistent over time and/or across situations, Réale, Reader, Sol, McDougall, & Dingemanse, 2007) is developing rapidly, and studying the causes and consequences of personality has become a major topic in behavioural and evolutionary ecology (Wolf, van Doorn, Leimar, & Weissing, 2007). Of the five major personality dimensions (Réale et al., 2007), the exploration–neophobia gradient is one of the most studied (Dingemanse, Both, Drent, van Oers, & van Noordwijk, 2002; Dingemanse & de Goede, 2004; Greenberg & Mettke-Hofmann, 2001; Verbeek, Drent, & Wiepkema, 1994) and has been investigated across a wide range of taxa (birds: Greenberg, 1983; Sol, Timmermans, & Lefebvre, 2002; rodents: Reader & Laland, 2003; monkeys: Day, Coe, Kendal, & Laland, 2003). Neophobia is defined as the avoidance of novel stimuli in the environment because these stimuli have never been encountered before and differ from stimuli that have been experienced in the past by the focal individual. This fear reaction is characterized by physiological and behavioural responses (Greenberg & Mettke-Hofmann, 2001) which rank the individual along the 'neophobia–neophilia' continuum and, hence, are linked to its capacity to explore novel environments (Réale et al., 2007).

Neophobia is one of the most studied personality traits, and is often considered to be part of a behavioural syndrome (i.e. correlated with other personality traits; Sih et al., 2004). For instance, in domestic mammals, aggressive individuals are fast and superficial explorers (i.e. neophilic) compared to nonaggressive individuals which are slow and thorough explorers (i.e. neophobic, Benus, Den Daas, Koolhaas, & van Oortmerssen, 1990; Benus, Koolhaas, & van Oortmerssen, 1987; Hessing, Hagelso, Schouten, Wiepkema, &

* Correspondence and present address: C. Monestier, Department of Animal Biology, University of Illinois at Urbana-Champaign, Champaign, IL 61801, U.S.A.
E-mail address: monestier.chloe@gmail.com (C. Monestier).

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Vanbeek, 1994). Individual differences in behavioural tactics are especially pronounced in stressful situations involving novel environments, conflict or inescapable shocks. For example, in great tits, *Parus major*, fast explorers approached a novel object faster than shyer individuals (Verbeek et al., 1994) and returned sooner to a feeding table than slow explorers (van Oers, de Jong, Drent, & van Noordwijk, 2004; van Oers, Drent, de Goede, & van Noordwijk, 2004).

Animal personality is particularly difficult to study in the wild given the difficulty of obtaining repeated measures of individual behaviour under standardized conditions (Campbell, Weiner, Starks, & Hauber, 2009). Consequently, most previous analyses of personalities have been carried out in captivity (see Herborn et al., 2010). Neophobia has been commonly assessed by manipulating the feeding environment using novel food, containers or objects placed near food. Indeed, several empirical studies have recently shown that neophobia impacts feeding behaviour (Herborn et al., 2010; King, Williams, & Mettke-Hofmann, 2015; Richard et al., 2008). Neophobia has been measured in terms of the time taken to approach and manipulate the object, the duration and frequency of investigation (Greenberg, 1983; van Oers, Drent et al., 2004; van Oers, Klunder, & Drent, 2005) or physiological measures (e.g. heart rate, Greenberg & Mettke-Hofmann, 2001). Studies on captive animals provide an informative first step towards understanding the link between personality and life histories in wild populations (Smith & Blumstein, 2008). Indeed, Herborn et al. (2010) found that an individual's exploratory tendency and level of neophobia measured in captivity successfully predicted the analogous traits measured in the wild.

We first aimed to investigate the existence of consistent individual differences in the level of neophobia among captive roe deer, *Capreolus capreolus*. Individual differences in behaviour have been linked to several life history traits of this species in the wild (Bonnot et al., 2015; Debeffe et al., 2014; Monestier et al., 2015). For instance, Debeffe et al. (2014) suggested that future dispersers were less neophobic (indexed by their exploration behaviour) than future philopatric individuals. Hence, we expected to observe consistent individual differences in neophobia with a standardized novel object experimental protocol of the type commonly employed for other taxa (Greenberg, 1983; van Oers, Drent et al., 2004; van Oers et al., 2005).

We then aimed to validate our approach for assessing individual differences in behaviour of roe deer in the wild. To that end, we used our experimental set up to explore the link between neophobia with the behavioural and biochemical measures that are routinely recorded during roe deer captures in both the wild and under captive conditions. Behavioural syndromes may be indexed by both physiological measures and behavioural traits due to the link between behaviour and the hypothalamus–pituitary–adrenal axis (Koolhaas, de Boer, Coppens, & Buwalda, 2010; Koolhaas et al., 1999). For instance, in birds, there is a rapid rise in glucocorticoids (Silverin, 1998), and a more marked increase in body temperature and heart rate following handling in shyer individuals than bolder ones (Carere & van Oers, 2004) linked to the 'emotional' stress response. We, thus, expected to observe a link between neophobia and other behavioural and biochemical responses to stress in captivity at the individual level. Behavioural differences under stressful conditions are generally interpreted within the 'coping style' (i.e. a coherent set of behavioural and physiological stress responses that are consistent over time and characterize a given group of individuals, Koolhaas et al., 1999) framework. Proactive animals are highly aggressive, take risks (i.e. are bolder) and explore more readily (i.e. are neophilic), whereas reactive individuals tend to freeze and are more generally passive (i.e. are shy and neophobic) in the face of potential danger (Groothuis, Müller, von Engelhardt, Carere, & Eising, 2005; Koolhaas et al., 1999). We previously showed that proactive deer exhibit more pronounced behavioural and physiological responses to stress than reactive animals (Monestier et al., 2016). Thus, we expected that the impact of a novel object on feeding behaviour would be especially marked among neophobic individuals that were characterized by the weakest low behavioural (behavioural score) and/or weak physiological responses (rectal temperature, level of haematocrit, proteins, fructosamine and neutrophil/lymphocyte ratio) to a stressful situation (i.e. capture event). In the light of this, given that personality in captivity may be assumed to reflect personality in the wild (Herborn et al., 2010), we would be able to reliably interpret individual differences measured in the wild in terms of a behavioural syndrome.

METHODS

Study Site and Population

The research station is in southwestern France, about 30 km southeast of Toulouse, on the slopes of a hill at around 230 m above sea level. The climate is of the 'Aquitaine' Atlantic type, although subject to a strong Mediterranean influence, especially in summer.

The captive roe deer live in nine permanently occupied enclosures of 5000 m^2 alone (territorial males only) or in groups of two to six according to their status (habituated or tame) and sex. In each enclosure, there is a cabin where individuals can shelter and where food is provided daily in the form of animal food pellets (Arterris cervis engraissement, 16% crude protein, 600 g per individual) in a feeder placed on the ground. Despite being habituated to human presence, the deer were able to express normal behavioural responses (e.g. vigilance) to stressful and/or novel stimuli, including disturbance within their familiar captive environment. Hence, we assumed that any interindividual differences in the behaviour of these captive animals would be indicative of natural behavioural variation and not a consequence of captivity.

Experimental Design

The experiment was carried out on 21 different individuals (five males: two juveniles and three adults; 16 females: five juveniles, six yearlings and five adults) and covered two consecutive winters (2014/2015, $N = 14$; 2015/2016, $N = 7$). During each winter, we carried out the experiments twice, once in November and once in February. We thus avoided the mating period and the late gestation/lactation period for females. For each session, the neophobia trial included two phases that were alternated over 10 days: a control phase with no disturbance (5 days with 7 h of tests per day) and a novel object phase (5 days with 7 h of tests per day). The novel object was placed above the feeder, slightly to one side so as not to inhibit all feeding. We used 10 different novel objects (two sessions on each of 5 days) to avoid habituation. The novel objects were geometric polystyrene shapes (circle, diamond, square, triangle) painted with contrasting colours, since roe deer are more sensitive to shapes and contrasts than to colours (Cohen, Osborn, Gallagher, Warren, & Miller, 2014).

On any given day (i.e. control phase and novel object phase), a few hours prior to observation, the feeder was removed to ensure that the deer would attempt to feed once the observations started. Then, at the start of observations, we put the feeder back with the usual feed, either with or without the novel object next to the feeder, before triggering a camera trap (Reconyx, HyperFire). The camera was fixed to the corner of the cabin opposite to the feeder to film the feeder, the novel object and the cabin trapdoor through which deer entered and left the cabin. The cameras were placed in