Animal Behaviour 83 (2012) 1295–1300

Contents lists available at SciVerse ScienceDirect

Animal Behaviour

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

Commentary

Do rapid assays predict repeatability in labile (behavioural) traits?

Peter A. Biro*

Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Australia

ARTICLE INFO

Article history:
Received 20 October 2011
Initial acceptance 11 November 2011
Final acceptance 18 January 2012
Available online 14 March 2012
MS. number: 11-00843R

Keywords:
acclimation
boldness
coping
endocrine
habituation
mixed model
personality plasticity
random regression
temperament

Laboratory studies of labile animal traits (e.g. physiology, behaviour) often place animals into a novel testing apparatus to do short-term assays, and later repeat the procedure to evaluate repeatability. Because animals in nature are never forced into these unnatural situations, measured values may not reflect those observed under more familiar (and therefore more natural) conditions. Thus, we implicitly assume rank order differences across individuals are maintained between these two contexts. I repeatedly assayed behavioural traits for young fish (Ward's damselfish, *Pomacentrus wardi*) in their home tanks, and observed significant repeatability once they were acclimated, but observations taken over the first 2 days did not predict behavioural types evident from subsequent observations. This cautionary note indicates that rapid assays of behavioural traits can significantly misclassify individuals. Furthermore, numerous physiological traits are often correlated with behaviour, suggesting caution for physiological studies as well. Future studies should not assume that labile trait assays predict scores under familiar conditions and, more importantly, should test whether scores under familiar laboratory conditions predict those observed in the field.

Although many biologists study animal traits in the laboratory, we ultimately want to understand the causes and consequences of individual trait differences that would be expressed under natural conditions. Studying how animals express labile traits (e.g. those related to physiology or behaviour) in the laboratory makes experiments more tractable, and provides control to isolate a given effect of interest. However, there are potential problems with this approach that seem to have gone largely unnoticed.

Studies often remove individuals directly from the field (Reale et al. 2000; Martin & Reale 2008; Boratynski & Koteja 2009), or from group housing in the laboratory (Ksiazek et al. 2004; Wilson et al. 2009), and then place them into a novel test apparatus to conduct short-term trait assays. Capturing the animals and then forcing them into novel (and presumably highly stressful) situations clearly differs from natural conditions. In nature, many animals can choose whether or not to expose themselves to novel conditions, and as a result they may often occupy habitats/situations that are usually familiar to them and that are not extremely stressful. When novelty is encountered in the wild (e.g. during dispersal, sudden appearance of a new predator), their response to it is not affected by artificially imposed stress, nor is their response constrained by unfamiliar and unnatural laboratory conditions. Therefore, the implicit assumption of any laboratory study of a labile trait, such as behaviour, is that its expression is a good predictor of trait

* Correspondence: P. A. Biro, Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, VIC 3216, Australia.
E-mail address: pete.biro@deakin.edu.au.

0003-3472/$38.00 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd.
doi:10.1016/j.anbehav.2012.01.036

expression of those same individuals under more familiar, less stressful, and therefore more natural conditions.

To evaluate a labile trait, researchers often repeatedly observe an animal's response to an assay for repeatability (i.e. consistent individual differences). Habituation (a decline in response to novelty or stress) and acclimation (a change in response while adjusting to novelty/stress) are common responses to repeated assays, occurring for hormonal (Romero 2004), physiological (Ellenberg et al. 2009) and behavioural traits (Budaev 1997; Romero 2004; Martin & Reale 2008; Wong et al. 2010). However, the assumption that trait levels measured under novel laboratory conditions are a predictor of those observed under familiar conditions will not be violated so long as individual acclimation responses are similar, and thus individuals maintain their rank order between novel and familiar conditions (Fig. 1a). However, if individuals differ significantly in the form of their acclimation response, the assumption is violated, and therefore rapid assays of labile traits will not predict those under familiar conditions (Fig. 1b). None the less, studies using rapid assays under novel conditions have clearly been informative about performance in the field (e.g. Reale et al. 2000; Boon et al. 2007). However, if the assumption is violated, then we may misclassify at least some of the individuals in a sample, and this could in turn affect our power to detect relationships between an individual's behavioural type and other variables of interest.

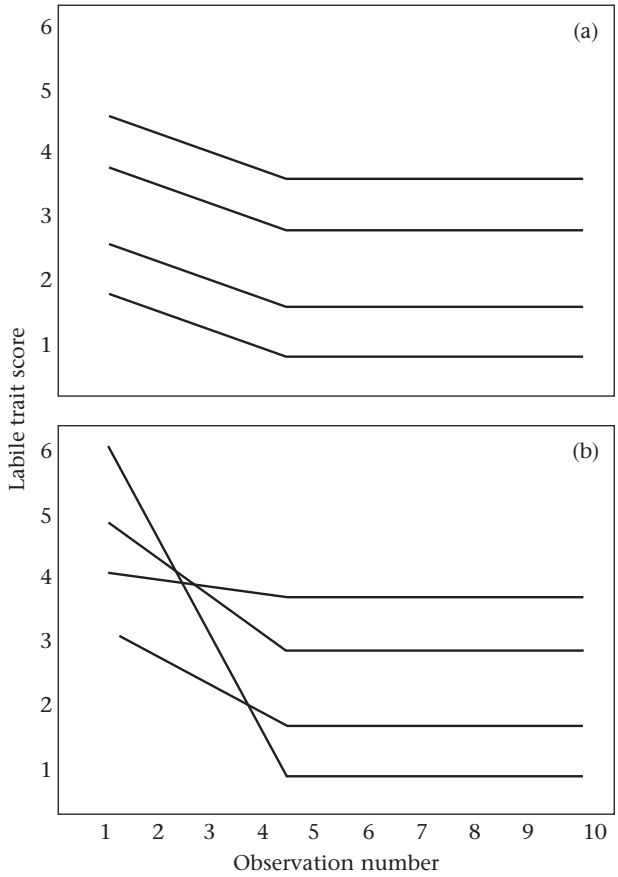

Figure 1. Hypothetical changes in a labile trait across repeated observations (e.g. locomotor activity or stress hormone response in an initially novel environment). (a) Individuals that consistently differ in their average levels of a trait, and follow a similar pattern of acclimation. In this scenario, even just a few initial (rapid) assays of naïve animals would yield a good estimate of the trait differences between individuals under familiar (and more natural) conditions, and we would detect significant repeatability both during and after the acclimation period. By contrast, (b) illustrates differences in acclimation, whereby the rank order of relatively naïve individuals would give an incorrect assessment of trait differences of animals under familiar conditions. In this second scenario, we would probably not detect repeatability during the acclimation phase, but would detect it after. Acclimation responses over time are illustrated as a two-phase process for ease of illustration, and to facilitate comparison with some of the results of the present study.

Surprisingly, it seems that this assumption has not been tested directly (but see Martin & Reale 2008; Rodríguez-Prieto et al. 2010, 2011). Perhaps this is so because testing the assumption would require numerous observations conducted on many individuals, and the need to span periods that include acclimation and post-acclimation intervals (Fig. 1). Indeed, studies rarely measure a labile trait more than twice to assess repeatability (Nespolo & Franco 2007; Bell et al. 2009), which is insufficient to test this assumption and might even explain why reported repeatability values are often low (Nespolo & Franco 2007; Williams 2008; Bell et al. 2009).

I repeatedly assayed behavioural responses of young fish housed in home tanks to test whether or not observations made under forced novel conditions predict behavioural traits under familiar and (presumably) less stressful conditions, conditions that are likely to be most similar to what animals in nature experience, most of the time. By extension, results from this study may also have relevance for a variety of physiological traits, such as metabolism and endocrine hormone levels, because they are often correlated with behavioural traits (e.g. Carlson 1986; Gosling 2001; Sih et al. 2004; Overli et al. 2005; Careau et al. 2008, 2010; Sih & Bell 2008; Williams 2008; Biro & Stamps 2010). This study thus represents an important first step towards determining whether rapid assays of labile traits are informative of what we might observe in the field.

METHODS

I performed the experiment in a temperature-controlled laboratory at Lizard Island Research Station, located on the northern Great Barrier Reef, Australia (14°41′S, 145°27′E). I captured large numbers (ca. 100) of larval Ward's damselfish, *Pomacentrus wardi*, that were in the process of settling to the reef, using light traps anchored just outside the reef crest (Meekan et al. 2001). Fish were caught overnight, and at dawn were brought back to the laboratory by boat in a large aerated bin, where they were held together in a 100-litre aquarium with fresh flow-through sea water at ambient temperature (ca. 28 °C) and live *Artemia* food until focal animals were selected later that morning (see below). At that point it was evident that all fish had undergone metamorphosis, indicated by the adoption of juvenile coloration and shape. Fish not used in the experiment were released at noon onto the reef adjacent to where they were captured. All research was conducted under permits from the Great Barrier Reef Marine Park Authority and James Cook University Animal Ethics Committee.

I randomly selected 30 individuals with similar body size (mean standard length = 12.9 mm, range 12.7–13.5 mm) and placed each fish into its own plastic aquarium by noon that same day. Each aquarium (25 × 16 cm and 17 cm high, filled to a depth of 10 cm) contained a layer of sand on the bottom and a small 'T'-shaped plastic pipe connector placed against the far wall, such that the three openings faced forward. Aquaria were visually isolated from one another and from the observer using plastic sheeting. Fish were fed recently hatched (<24 h old) live *Artemia* nauplii up to three times per day throughout the experiment to ensure ad libitum food, visually confirmed to be swimming about the aquaria. Without moving aquaria, I used a siphon to change 80% of the water at the end of every second day with fresh, temperature-adjusted sea water. Artificial light was provided on a 13:11 h light:dark regime matching outside light conditions. Fish were euthanized (confirmed by cessation of opercular beats for 5 min) using an