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Ecological and evolutionary significance of within-individual behavioural variation
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Background

Consistent between-individual behavioural differences across various ecological situations and over time in single (*animal personality*) or across multiple, functionally different behaviours (*behavioural syndrome*) has been a hot topic of evolutionary behavioural ecology in the last two decades (for reviews and meta-analyses see Bell et al., 2009; Dall et al., 2004; David & Dall, 2016; Dingemanse & Dochtermann, 2013; Kaiser & Müller, 2021; Sih et al., 2012; Smith & Blumstein, 2008; Stamps & Groothuis, 2010; Stuber et al., 2022; Weiss, 2018). While between-individual behavioural variation is traditionally approached by focusing on behavioural type (e.g., how aggressive an individual is on average), considerable research effort has been made to reveal the eco-evolutionary relevance of the within-individual behavioural variation too. As a result, behavioural plasticity (e.g., how much an individual's aggression varies as a result of environmental change) and behavioural predictability (e.g., how much an individual's aggression varies irrespective of the environment) are now recognized as valid and important components of an individual's behavioural strategy (see e.g., Stamps et al. 2012; Stamps 2016; Mitchell et al. 2021; Dochtermann 2023; Horváth et al. 2023).

In principle, behavioural plasticity, behavioural predictability, and behavioural type could be independent characteristics of an individual, as any behavioural type could occur with either high or low plasticity or predictability. However, another intriguing possibility is that within-individual behavioural variation is 'personality-dependent', i.e., behavioural plasticity and predictability are correlated to behavioural type through genetic or environmental links (see Dingemanse et al. 2010; Dingemanse and Wolf 2013; Mathot and Dingemanse 2014). In this scenario, selection on the behavioural type (e.g., increased boldness) would produce an indirect, correlated response in within-individual behavioural variation as well. In contrast to this notion, our recent phylogenetic meta-analysis reveals no general correlation between behavioural type and components of within-individual behavioural variation, suggesting that the link between behavioural type, behavioural plasticity and predictability is rather shaped by selection than physiological, developmental, or environmental constraints (Horváth et al. 2023). The project's

main goal was to gain a better understanding of the ecological and evolutionary significance of individual behavioural plasticity and behavioural predictability, as well as their connection to behavioural type.

Conceptual work: meta-analysis

At the time of submitting the proposal, we were working on a phylogenetic meta-analysis to test whether behavioural type and behavioural predictability evolve independently (lack of correlations), or their evolution is constrained (abundant correlations). In the latter scenario, correlations between the two traits may emerge due to either proximate constraints (the direction of correlations is similar) or local adaptations (the direction of correlations is variable). Our results support the adaptive scenario: correlations between behavioural type and predictability are abundant in nature, but their direction is variable. We suggest that the evolution of these behavioural components might be constrained in a system-specific way. (Horváth et al. 2023)

Testing evolutionary adaptations of within-individual behavioural variation

Intuitively, in more stable environments (i.e., caves with no light, stable temperature, and extremely simple communities) decreased behavioural flexibility is expected to be beneficial since maintaining the ability to express plasticity and the expression itself has considerable costs (Dingemanse and Wolf 2013; Mathot et al. 2012; Sih 2013). Additionally, high behavioural predictability in stable environments is expected. To test these questions, we compared the behaviour of cave-adapted and surface-dwelling populations of the common water louse (*Asellus aquaticus*) in laboratory settings. Our results reveal a complex picture of how cave adaptation affected various behavioural traits in this species.

Regarding feeding behaviour, we assayed all populations with the familiar and unfamiliar food types from the natural habitats and two novel food types not occurring in the natural habitats of the species. We found that all populations (surface and cave alike) preferred surface to cave food and consumed the unnatural novel food types. Therefore, we conclude that the cave population maintained its preference for surface food and did not lose its food type innovativeness (i.e., behavioural flexibility), contrary to our expectations (Herczeg et al. 2021).

In a second experiment we tested for variation in aggregation and sheltering behaviour in cave- and surface-dwelling *As. aquaticus* populations. While we found high inter-population

variation in both behaviours, this difference was not explained by habitat type (Horváth et al. 2021). In a separate experiment, we assayed the risk-taking behaviour (i.e., freezing latency after a simulated predator attack) between the populations, but similarly to the patterns described above, we did not find clear effect of habitat on the presence/absence/strength of personality (i.e., repeatability) or on behavioural type (Berisha et al. *in prep.*)

Movement activity in different populations of *As. aquaticus* was targeted by several experiments. In a pilot study, we detected increased movement activity in cave-adapted individuals, especially, males (Berisha, Horváth et al. 2022), most likely due to relaxed predation pressure in the cave-adapted population. Nevertheless, in another study, where we repeatedly tested dispersal-related novel area exploration and dispersal speed in cave- and surface-adapted populations, surface populations were more explorative and dispersed faster than their cave conspecifics, suggesting that extreme and isolated habitats like caves might work as 'dispersal traps' following successful colonisation because adaptation to these habitats includes the reduction of exploratiVeness. Furthermore, our results suggest that individuals with higher exploratiVeness are likely to colonise markedly new environments (Horváth et al. 2023).

To examine the effects of surface versus cave environment on behavioural means and variation, we implemented a longitudinal experiment in which we assayed activity and boldness of *As. aquaticus* from multiple cave- and surface-adapted populations. We found that both within- and between-individual variations in movement activity were lower in cave than in the surface habitats. More active or bolder individuals tended to be more plastic. The direction and strength of light-induced plasticity were more variable in surface than in cave individuals, resulting in lowered detectable group-level plasticity in surface populations (Kralj-Fišer et al. *in prep.*). Nevertheless, we suggest that other cave-surface systems and other (e.g., morphological, physiological) traits should be studied to see whether the patterns are general.

How does selection on behavioural type affect within-individual behavioural variation?

Clarifying how strongly are the three components of individual behaviour (type, plasticity, predictability) linked is a primary goal in understanding how stable between-individual behavioural differences within a population emerge. If behavioural type influences plasticity and predictability, there is no space for independent evolution of the latter (Mathot and Dingemanse 2014). However, if the three components are independent, stable between-individual differences might not only evolve due to selection favouring divergent behavioural

types but also due to selection favouring high predictability. Somewhat similarly, genetically based between-individual behavioural correlations (behavioural syndrome) constrain the independent evolution of different behaviours (see Sih et al. 2004a, b; Dochtermann and Dingemanse 2013).

We performed two laboratory experiments with *P. reticulata* lines selected for divergent behaviour types. These selection lines vary significantly (> 15% difference) in key social behaviours such as collective activity and group cohesion (see Szorkovszky et al. 2018). We wanted to test whether artificial selection on the individual mean behaviour (behavioural type) of one behavioural trait, that is schooling behaviour (= sociability) affects the behavioural type, behavioural plasticity and behavioural predictability in other behavioural traits. In the first experiment, we used males only and tested their aggression towards conspecific males and courtship behaviour towards conspecific females, while in the second experiment movement activity and risk-taking (time spent in 'open water') in adult females were assayed.

To date, two manuscripts are in preparation (Sztruhala et al., *in prep*). Our preliminary results indicate that females from different selection lines do not differ either in their behavioural type or behavioural predictability, indicating that selection on sociability behavioural type does not affect behavioural variation in other behavioural traits (namely, activity and risk-taking). This result is somewhat in line with the outcome of our meta-analysis (see above), in which we found that in 44% of assessed cases, behavioural type and behavioural predictability are independent (Horváth et al. 2023), i.e., there is no general correlation between behavioural predictability and behavioural type.

Phenotypic plasticity of within-individual behavioural variation

If we accept that behavioural plasticity (i.e., the direction and magnitude of behavioural change induced by environmental change) and behavioural predictability (i.e., within-individual behavioural variation unrelated to the environment) are valid phenotypic traits of an individual, they might also be phenotypically plastic (viz., the ability of a genotype to produce different phenotypes as a response to environmental variation). For 'plasticity of behavioural plasticity' see e.g., Urszán et al. (2018). Preliminary results show that individuals generally behave unpredictably in situations with elevated predation pressure (see Stamps et al 2012; Briffa 2013; Nakayama et al 2016; Horváth et al. 2019).

Using common pill bug (*Armadillidium vulgare*) as a model species, we conducted multiple long-term laboratory experiments to test how variability in abiotic environment components affect behavioural type, plasticity and predictability.

In the first experiment, we had two treatment groups acclimated at a constant 25 C°, while the other experienced daily changing temperature (25 C° vs. 31 C°). Both groups' behaviour (i.e., startle response, a proxy of risk-taking) was tested repeatedly at both temperatures. We expected that individuals in the stable temperature group would become less bold and less predictable at higher temperatures, while such substantial differences were not expected for the other group as they acclimated to both temperatures. Our preliminary results partially support our expectations: temperature affected only behavioural type in the stable temperature group, while we found sex-specific differences in how animals react to different temperatures (Sztruhala et al. *in prep.*)

In a second experiment, we tested whether microplastic (particles < 5mm) pollution affects behavioural variation of *Ar. vulgare*. Since to date the effect of microplastic pollution on personality was tested only on short-term (~ one week), we implemented a long-term (two months) experiment. Briefly, after we determined the baseline behaviour (five repeats), we treated half of the animals with microplastics for one month and tested their behaviour (five repeats) again to assess potential differences between the treatment and control group. Preliminary results show that risk-taking significantly decreases in the microplastic-treated group, while not in the control group (Horváth et al. in prep.)

Miscellaneous

During the funding period, we purposefully aimed to broaden the scope of the project as much as possible and incorporate any new ideas that would help us in reaching our research goals.

Building on our already existing research with Carpetan rock lizard (*Iberolacerta cyreni*), we aimed to integrate thermoregulatory strategy into animal personality framework. In line with previous experimental studies (Goulet et al. 2017a, b; Michelangeli et al. 2018), we found a moderate link between risk-taking personality and behavioural thermoregulatory strategy in this species (Horváth et al. 2020a). More recently, in common lizard (*Zootoca vivipara*) we found animal personality to be present in both 'classic' behavioural traits and different thermoregulatory traits (selected body temperature, voluntary thermal maximum, setpoint

range). Besides an activity–risk–taking syndrome, we also found a risk–taking–selected body temperature syndrome (Horváth, Sos et al. *in prep.*)

We also tested the effect of environmental stress on various components of behavioural variation in *I. cyreni*. From an ecological point of view, stress can be defined as an increase in one or multiple environmental factors that force the organism out of its ecological niche. In theory, extreme stress (e.g., severe drought) may force individuals to follow a single behavioural strategy, thus, canalising behavioural variation. Conversely, when stress doesn't pose immediate negative effects on survival (e.g., mild hunger), diverse individual behavioural strategies may emerge. In a pilot study, we found moderate-high repeatability in antipredator aggression (willingness to bite a human), indicating the presence of animal personality in this behavioural trait. Lizards were on average more defensive in the presence of predator cues (i.e., mild stress); furthermore, short-legged males showed higher antipredator aggression than long-legged males in the presence of predator cues, probably as an attempt to balance their decreased escape speed. This indicates that antipredator aggression is an important part of an individual's behavioural repertoire, and its expression is driven by both environmental situation and individual state. (Horváth et al. 2020b). Further, results from another, longitudinal study on the species show that the presence of predator cues induces the emergence of behavioural consistency, in line with the diversifying scenario. We found no/ weak link between behavioural type/ predictability and individual state. Both behavioural type and predictability show consistent differences before and after treatments, but the correlation between them vanishes due to treatments (Horváth et al. *in prep.*).

In collaboration with the Hungarian meadow viper LIFE project, we initiated a research project to investigate how captive breeding affects the personality (feeding behaviour, activity and boldness) of the endangered *Vipera ursinii rakosiensis*. Our preliminary results show that limited environmental complexity potentially in interaction with low genetic variation resulted in rather low repeatability (i.e., personality) in the studied behaviours, which may pose problems in repatriated populations (Halpern et al. *in prep*)

Lastly, in collaboration with researchers from the Department of Ecology of the University of Szeged, we studied how habitat types affect individual and group behavioural variation in different *Myrmica* species. Our results suggest that habitat type, through its environmental characteristics, can affect different behavioural traits both at the individual and colony level and

that those with the strongest effect on colony productivity primarily shape the personality of individuals. Further, there is a need for complex environmental manipulations to fully understand the effects shaping behaviour and reproduction in colony-living species (Maák et al. 2021, Maák et al. *in prep*).

Summary and future prospects

Overall, the funded project was a success. Despite an initial delay due to the COVID-19 outbreak, we were able to reach the planned goals, further, we could incorporate several studies that were not planned at the time of applying. To date, we published eight papers in high-ranked journals like *Behavioural Ecology and Sociobiology*, *Behavioral Ecology*, and *Royal Society Open Science*. Currently, we have several more manuscripts in preparation connected to the project. We also published one paper in *The Herpetological Bulletin* that was only loosely connected to the proposed project, but where the funding still helped. We also presented our results at numerous national and international conferences (e.g., ISBE 2022, ABS 2023).

In addition to publishing the scientific results, the educational string of the project was also strong. Sára Sztruhala, who works as a PhD student (under my co-supervision) on the biological importance of within-individual behavioural variation, is expected to defend in two years.

During the project, our main collaborators were Prof José Martín (National Museum of Natural Sciences, CSIC, Spain), Prof Cene Fišer (Department of Biology, University of Ljubljana, Slovenia) and Prof Niclas Kolm (Department of Ethology, Stockholm University, Sweden). In Hungary, my main collaborator was Prof László Zsolt Garamszegi (Centre for Ecological Research, Institute of Ecology and Botany). During the project, we started a very promising collaboration with the Pavol Jozef Šafárik University (Igor Majláth; Košice, Slovakia), the Babeş-Bolyai University (Tibor Sos, Péter László Pap; Cluj-Napoca, Romania) and the Hungarian meadow viper LIFE project (Bálint Halpern; Kiskunság National Park, Hungary).

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