

Behavioural consistency in agile frog tadpoles (*Rana dalmatina*)

RÉSUMÉ OF DOCTORAL THESIS

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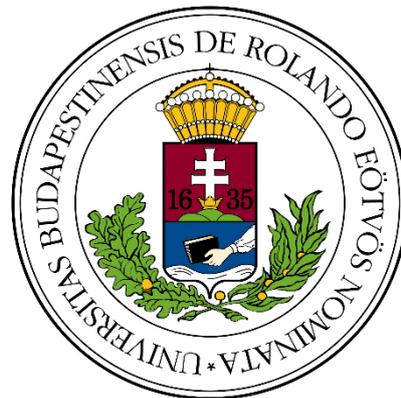
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Budapest
2015

1. Introduction

According to a general definition, personality is the collection of traits that describe and explain the temporary consistent patterns of cognition and behaviour of individuals (Gosling 2008). Personality has fascinated humans since the dawn of time, but its scientific recognition in animals is relatively young. After the pioneering studies of Huntingford (1976) and Clark and Ehlinger (1987) on animal personality it has recently become a hot topic in evolutionary behavioural ecology with the aim to understand the evolutionary and developmental mechanisms behind the emergence of consistent individual differences in animal behaviour. Behavioural consistency has two levels; animal personality are repeatable individual differences within behaviours while behavioural syndrome are correlations between functionally different behavioural traits (Garamszegi and Herczeg 2012; Jandt *et al.* 2014). Behavioural consistency have been observed in the wild in a wide range of taxa from Anthozoa to Mammalia (Smith and Blumstein 2008; Garamszegi *et al.* 2012, 2013). Throughout the years many have assessed the ecological and evolutionary implications of the different levels of behavioural consistency (Sih *et al.* 2004a,b, 2012; Bell 2007; Wolf & Weissing 2012; Dochtermann & Dingemanse 2013), which in turn led to numerous hypotheses aiming to explain the prevalence and origin of this phenomenon (Stamps 2007, Wolf *et al.* 2007, Dingemanse & Wolf 2013, Sih *et al.* 2015).

Behavioural consistency might seem maladaptive at first glance since it constrains individual behavioural repertoire through the existence of consistent individual differences in personality and constrains the evolution on functionally different but correlated traits through the existence of behavioural syndromes since selection on a given trait will affect others part of the correlation structure. For example in the fishing spider (*Dolomedes triton*) excessive precopulatory cannibalism have been detected: some females don't mate at all as they cannibalize all approaching males. Despite the fact that these spiders don't produce offspring this phenomenon emerges again in the next generation (Arnqvist and Henrikson 1997). By using the framework of behavioural consistency it has been found that juvenile voracity correlates with that of an adult's, juvenile voracity also correlated with aggressiveness and boldness under a simulated predator attack (Johnson and Sih 2005; 2007). The authors have argued that selection favours aggressive, bold and voracious juveniles to increase survival and dispersion but through behavioural spill over this causes lower fecundity in adult females. The

constraint created by the correlation between different behaviours lasting through life stages can create complex patterns of evolution resulting in selection favouring and selecting against voracious, bold and aggressive spiders (Mather and Logue 2013). Behavioural consistency can also be apparently adaptive. For example in three-spined sticklebacks (*Gasterosteus aculeatus*) behavioural syndromes only exist in populations under predation pressures (Bell 2005; Dingemanse *et al.* 2007). An experiment carried out by Bell and Sih (2007) found that after introducing a predator to individuals from a predator free population the survivors show the emergence of the behavioural syndrome. There is also evidence that some configurations of behavioural consistency can result in higher fitness (Biro and Stamps 2008) which was found even in humans (Eaves *et al.* 1990).

Numerous hypotheses have emerged, that try to explain the high prevalence of behavioural consistency. Studies on stress responses on laboratory animals have found numerous connections of consistent individual differences in coping to stress along the proactive-reactive axis to underlying physiological and genetic differences (Coppens *et al.* 2010; van Oers and Mueller 2010). These differences have been found in pigs (Hessing *et al.* 1994), in birds (Groothuis and Carere 2005), in primates (Kalin and Shelton 2003) and even in fish (Øverli *et al.* 2007). These findings coupled with quantitative genetic studies on behavioural traits imply the presence of genetic background of behavioural consistency and led to the emergence of the constraint hypothesis (Sih *et al.* 2004b; van Oers *et al.* 2005, van Oers & Mueller 2010, Dochtermann & Dingemanse 2013). According to the ‘constraint’ hypothesis behavioural consistency forms by individual differences in underlying proximate mechanisms like physiology (for example a single hormone affecting multiple behaviours Ketterson and Nolan 1999) genetic base, linkage and pleiotropy (van Oers *et al.* 2005; van der Waaij *et al.* 2008; Dingemanse *et al.* 2009; van Oers and Mueller 2010; Dochtermann and Dingemanse 2013; Rigterink and Houpt 2014) or temperament (existence of psychological constraints Gosling 2001; Garamszegi *et al.* 2013). If the constraint hypothesis would be true there wouldn’t be population differences in behavioural consistency in a given species. Studies on the other hand have found that populations of the same species show presence or absence patterns of behavioural consistency congruent with the strength of predation risk (Bell 2005; Dingemanse *et al.* 2007) which lead to the emergence of the ‘adaptive’ hypothesis (Sih *et al.* 2004b, Bell 2005) which states that behavioural consistency forms as local adaptation to the environment. However phenotypic plasticity (the ability of a single genotype to produce different phenotypes induced by environmental variation, West-Eberhard 2003) via direct environmental induction might also be responsible for the different patterns

observed. Behavioural syndromes can be induced in predator-naive individuals originally lacking the syndrome by exposing them to predation risk (Bell and Sih 2007), and environmental complexity in general is a key factor in the formation of behavioural syndromes (Sweeney *et al.* 2013). It has also been found, that even in selection lines selected for personality differences the environment plays a major factor in the emergence and shifting of behavioural consistency (Groothuis and Maestripieri 2013). By changing the caring behaviours of mothers their young later show shifted personalities resembling that of other selection lines (Curley and Branchi 2013; Schuett *et al.* 2013). There is also evidence that experience during ontogeny affects behavioural syndromes in laboratory mice (Benus and Henkelman 1998).

Other models explain the emergence of behavioural consistency by suggesting the existence of feedback loops between consistent behavioural strategies and state variables which operate through correlational selection (Sih *et al.* 2015). One of the most complex models of these feedback models is the Pace-of-Life Syndromes (POLS) hypothesis, which integrates behaviour, cognition, physiology and life history (Biro and Stamps 2008; Réale *et al.* 2010; Sih and Del Giudice 2012; Sih *et al.* 2015). To explain the adaptive nature of behavioural consistency the Pace-of-Life Syndromes hypothesis describes POLS along a fast – slow lifestyle continuum (Réale *et al.* 2010). The POLS hypothesis states that behavioural types and life history characteristics can couple in certain specific combinations, that can be more adaptive in different environments than others, but in a heterogeneous environment these various strategies may yield equal expected life-time fitness (Biro *et al.* 2006; Réale *et al.* 2010; Wolf and Weissing 2010). In this view, behavioural consistency arises from variation of life-history strategies between individuals in a population (Stamps 2007; Wolf *et al.* 2007; Careau and Garland 2012). The fast pace-of-life strategy incorporates individuals that are expected to be more active, risk-taking, have low social activity, are more aggressive and to explore more superficially. These individuals are also expected to have earlier maturity, faster metabolism and weaker immune system, to be routine forming, to be good at learning new things and have lower information processing capabilities. On the opposite end of the continuum, slow pace-of-life individuals are expected to be shy, less active, highly social, less aggressive and slow but thoroughly exploring. These also should have longer life span, longer developmental time, more efficient immune responses and more advanced cognitive apparatus, be better at reversal learning and information processing (Réale *et al.* 2010, Sih and Del Giudice 2012). More and more surveys are accumulating that try to test the various predictions of the POLS hypothesis, but so far the results have been mixed (Careau *et*

al. 2011; Niemelä *et al.* 2012; David *et al.* 2012; Adriaenssens and Johnsson 2013; Hulthén *et al.* 2013; Le Galliard *et al.* 2013; Løvlie *et al.* 2014; Cole and Quinn 2014). Although most of these studies conducted in various taxa were supportive not one study found all the predictions at the same time, and in some cases contradictions emerged.

Behavioural consistency is a group level phenomenon which requires multiple measurements of different behaviours in a group of individuals. Repeatability calculations are used to assess the consistency of each behaviour of interest. Animal personality in given behaviours can be numerically attributed to individuals by calculating behavioural types. Behavioural syndromes are detected by searching for correlations between functionally different behaviours – which cannot be numerically attributed to individuals directly. One can use dimension reduction or other more direct methods (Herczeg and Garamszegi 2012) to gain individual estimates of behavioural syndromes. One should also be aware that correlations apparently representing a behavioural syndrome can form from within and/or between individual correlations, and only the latter is the true indicator of a behavioural syndrome (Dingemanse and Doctermann 2013; Dingemanse and Réale 2013). By placing an individual into a set up with one increasing environmental gradient one can assess its behavioural reaction norm, where the slope of the fitted line represents the behavioural plasticity of the given individual which numerically represents the plastic response in a given behaviour to the studied environmental gradient (Dingemanse *et al.* 2010; Dingemanse and Wolf 2013). When placing individuals in standardised conditions and subjecting them to multiple behavioural measurements individuals still statistically differ in their variation around their specific behavioural types meaning that some of them are more consistent in their own behaviour than others (Stamps *et al.* 2012). This behavioural predictability (Biro and Adriaenssens 2013) have been recently found to be an important component along behavioural type to represent an individual's personality (Wesneat *et al.* 2012; Briffa 2013; Briffa *et al.* 2013; Wesneat *et al.* 2014). Individual numerical representations of all these traits are essential if one wants to study how behavioural consistency relates to fitness and ultimately its evolution.

2. Thesis objectives

In my thesis I have aimed to study behavioural consistency in agile frog (*Rana dalmatina*) tadpoles from a population under high predation pressure. Amphibians are mostly neglected in behavioural consistency research, however their larval stage presents a unique opportunity to study the emergence of personality during ontogeny and its possible link to fitness variables.

1. By using a common garden approach (all individuals are subjected to the same stimuli under laboratory conditions, the sole differences being in treatments) I studied the emergence of behavioural consistency in predator and conspecific naïve tadpoles. If tadpoles show personality under such conditions then it is possible that personality has a strong genetic component in the studied population.

2. By testing behavioural consistency in two tadpole life stages it is possible to assess the stability or change of personality during ontogeny. It is highly likely that personality is stable between younger and older tadpoles, as it was detected between older tadpoles and newly metamorphosed individuals in another study (Wilson and Krause 2012). This approach is also able to test if standard behavioural surveys conducted on the younger stage have any effects on the personality of the later tadpole stage. I predict that standard measurements won't have any effects on the behaviours of later larval stages.

3. By using the Pace-of-Life Syndrome (POLS) framework I predict various correlations between the studied personality traits of tadpoles and indirect fitness predictors. More active, risk-taking and superficially exploring individuals should reach metamorphosis faster and they should achieve higher relative mass gain during ontogeny.

4. By assessing individual behavioural predictability I predict the possibility of the integration of behavioural predictability into the POLS framework, if correlations form with indirect fitness predictors.

5. By presenting tadpoles with relevant stimuli (predator and conspecific presence) in during ontogeny in a full factorial common garden experiment, it is possible to survey the importance of individual experience and phenotypic plasticity in the emergence of behavioural consistency. I predict that both stimuli will have significant effects on the emergence of behavioural consistency.

3. Materials and methods

The agile frog (*Rana dalmatina*) is the most common brown frog in Hungary. The study population is located on the Island of Szentendre, where it uses multiple breeding habitats. One of them is located near Szigetmonostor situated in the floodplain of the Danube. The interconnected lakes here have variable water levels depending on the river, and flooding supplies them with fish. There are also many invertebrate predators present, and multiple species of anurans use the ponds for breeding.

1st experiment – objectives 1-4

Eggs were collected on the 17-20 of March in 2011. A small amount of eggs were collected from 80 clutches and brought into the laboratory where egg size was recorded for each clutch using 10 random eggs photographed by digital camera with reference and using the open source UTHSCSA Image Tool v. 3.0. A number of 30 eggs from each clutch were placed in plastic containers (8 litre volume, 34 × 23 × 16 cm) with two litres of RSW (reconstituted soft water, APHA 1985) and a black grid glued on the outside of the underside of the container to facilitate movement recordings. The containers were placed in a way which prevented visual contact of adjacent tadpoles and were kept in a laboratory with constant 19 °C temperatures and a 12:12 light cycle. When the 30 eggs hatched one healthy looking individual was left in each container. Tadpoles were fed *ad libitum* with boiled spinach and had their water changed every 4 days. Tadpoles were divided into two groups: the first group only to have their behavioural measurements taken when reaching Gosner stage 32-36 (beginning of toe development, Gosner 1960) named the ‘naïve group’; the second group also having behavioural measurements at a younger age of 11 days old, named the ‘disturbed group’. This set up also enabled me to study the young tadpoles of the latter as a group named ‘11 days old’. I have chosen three personality traits to be assessed: activity in a familiar environment (in this case the home container); exploration of an unfamiliar environment (exploring a novel large container); risk-taking in a familiar environment (a simulated predator attack in the home container). Behavioural trials lasted for three days and all three personality variables were assessed every day, resulting in three repeated measures of each trait in a given tadpole stage. Behaviours were recorded by a web camera system consisting of multiple cameras and laptops and using the open source Dorgem software with circa 1 frame / second rate. On any given trial day activity was assessed first, and then risk-

taking or exploration randomly. Between the latter two trials tadpoles were left to rest for two hours. Activity was assessed for 30 minutes, and movement frequency was calculated by dividing frames spent moving with all available frames. Exploration was assessed in an unfamiliar environment consisting of a large container with $80 \times 32 \times 18$ cm dimension and holding 4 liters of RSW. The area of the container was divided into 40 rectangles and had four obstacles to prevent overseeing of the whole area. Tadpoles were carried with a net two this container and were placed behind a lid for 5 minutes of acclimatization – after this the lid was lifted and tadpoles were left exploring for 25 minutes. Exploration was calculated by dividing the number of rectangles visited with the total number of traversable rectangles. To assess risk-taking a standardised predator attack was simulated by a customised tube letting a metallic rod fall into the water near the target tadpole. Flee response and latency time was recorded for 15 minutes. Risk-taking was calculated by counting the time taken for any tadpole to resume moving after a simulated attack-flee-freeze latency response. When tadpoles neared Gosner stage 42 (the emergence of the hind limbs) they were checked every 3 hours. Upon reaching the aforementioned stage and thus beginning metamorphosis I assessed individual developmental time taken to reach this stage and measured individual weight.

Data analysis

I have only analysed those animals that have reached Gosner stage 42. Due to developmental anomalies, data loss during recording and accidents during handling only half of the tadpoles could be used. I have excluded some individuals from analyses including risk-taking as in a few cases 15 minutes were not enough to record the whole latency time. These cases received the maximal score, which in turn could create false repeatabilities and predictabilities for individuals with multiple maximal scores for repeated measures.

Following Becker (1985) based on variance analysis I have conducted repeatability calculations on all three behaviours separately in each group of tadpoles using the available three measurements per personality trait. I have used general linear mixed models (GLMM) to detect changes in behavioural consistency during ontogeny by focusing on the individual \times ontogenetic stage interaction.

To detect possible behavioural syndromes I have applied Spearman correlations on the calculated behavioural types (here: mean) of repeatable behaviours. Regardless if a given behaviour was repeatable or not, I have included behavioural predictability (here: standard error) of all surveyed personality traits in the correlations to search for complex behavioural

strategies. To control for the large number of possibly non-independent tests I have applied false discovery rate (FDR) corrections (Benjamini és Hochberg 1995). I have also applied principal component analysis (PCA) on the dataset of the ‘disturbed group’ when a trend of a behavioural syndrome could be seen in the results of Spearman correlations.

For the detection of possible pace-of-life syndromes (POLS) I have used generalized linear models (GLM) with backwards stepwise selection method in all the studied groups, with repeatable behavioural types and behavioural predictabilities for all recorded behaviours as covariates. The dependent variables were developmental time taken till reaching metamorphosis (Gosner stage 42) and relative mass gain (gained by entering developmental time as a covariate into the models). Egg size was used as a proxy for maternal effects in all of the models.

2nd experiment – objective 5

Eggs were collected from 21st of March till 8th of April in 2013 from the same set of ponds as in 2011. This time the eggs were delivered to the laboratory of the “Lendület Evolutionary Ecology Research Group”. I have also captured six late instar dragonfly larvae (*Anax imperator*) on the site and the “Lendület group” supplied me with a juvenile pike (*Esox lucius*). Both predators were kept in RSW and were fed with tadpoles to gain predator presence stimulus. 40 clutches were sampled and some eggs were reared in large containers to present enough tadpoles to feed the predators and to have a large number of larvae to use for conspecific presence stimulus. The full factorial treatment consisted of 4 groups, in which 30-30 eggs were divided from each clutch. When the tadpoles hatched one haphazardly selected healthy tadpole was left the ‘control’ and ‘predator only’ groups, while five-five tadpoles were left in the ‘conspecifics present’ and ‘predator and conspecifics’ groups respectively. All tadpoles were kept in a common garden setup, in a laboratory with 19 °C and a 12:12 light-dark photoperiod, with *ad libitum* frozen and later boiled spinach supplied as food. The tadpoles taking part in the experiment were kept in non-transparent, white 1.5 L plastic containers (20.6 × 14.6 × 7.5 cm) filled with 0.8 litres of RSW. During ontogeny all groups received stimulus water administered from syringes two times a day. Per administration the ‘control’ group received 40 ml RSW; the ‘predator only’ group received 40 ml stimulus water consisting of 20 ml pure RSW and 20 ml water taken from dragonfly and pike predators in a 1:1 ratio; the ‘conspecific present’ group received 40 ml stimulus water consisting of 20 ml

pure RSW and 20 ml of water taken from large tanks filled with conspecifics; the ‘predator and conspecifics’ group received 40ml stimulus consisting of 20ml water taken from conspecific tanks and 20ml water taken from predator tanks mixed in a 1:1 ratio. The tadpoles were left to develop until reaching Gosner stage 32-36 (beginning of toe development) when behavioural assays began. Every tadpole had its water changed before the trials and in the treatments with multiple tadpoles present one tadpole was selected haphazardly and left in the container and others were removed. For one day stimulus water was administered as usual, and on the next three days trials were run. During each trial day activity and risk-taking were assessed using web cameras and laptops in the same way as in 2011. Activity was measured in a familiar environment, stimulus water was administered first and after 5 minutes activity was recorded for 30 minutes. Activity was calculated as the distance crossed in millimetres during the recording time with the help of Gergő Nagy and MATLAB. After two hours stimulus water was again administered and 5 minutes later a I inflicted a simulated predator attack by poking the tadpoles tale with a small paintbrush and recorded the individuals for 35 minutes. Latency time to resume normal movement after a time spent freezing was counted in seconds with the help of Gergő Nagy.

Data analysis

Note that in the experiment presented here only the half of the tadpoles could be used, as the others were subjected to different trials. There was some minor loss of individuals due to developmental anomalies or death.

I ran general linear mixed models (GLMMs) on the two behaviours using REML estimation in the R statistical environment (lme4 package) to assess if treatments had any effect on mean behaviour. Group-specific repeatability estimates for personality traits were gained from GLMMs fitted separately on each subset data of different treatments. By using extracted components I calculated the proportion of the between-individual variance relative to the total variance (Nakagawa & Schielzeth 2010) for each behaviour in each group.

To statistically decompose the between and within individual effects which is necessary for detecting valid behavioural syndromes, I used Bivariate Mixed models (MCMCglmm package) created by László Zsolt Garamszegi following Dingemanse and Dochterman (2013). By relying on long iterations and by running each model at least four times I took 1000 samples from the gained Markov Chains to obtain the parameters of interest and to gain the 95% credibility intervals around them. As a contrast analysis I have calculated

the phenotypic correlations between traits by using classical approaches as an illustration of the importance of the separation of correlational components and the usage of bivariate mixed models.

4. Theses

4.1 – Behavioural consistency and POLS in predator and conspecific naïve tadpoles

1. I have detected that tadpoles kept in conspecific and predator free common garden environment develop repeatable personalities. Only activity was repeatable in both younger and older tadpoles. Exploration was only repeatable and thus became a personality trait in older tadpoles. Risk-taking was only consistent in the ‘disturbed group’ which was previously tested at a younger age. These findings suggest the importance of ontogenesis in the formation of consistent individual differences in behaviour. It is also apparent that risk-taking only became repeatable in an older tadpole group after receiving the stimulus of standardized tests conducted at a younger age which suggest a high susceptibility to environmental induction during ontogeny.

2. I have detected that during ontogeny the repeatability of activity decreased, as younger tadpoles were more consistent than older ones. Only the behavioural type of exploration showed a clear increase as older tadpoles were able to explore more areas, but this might have been due to size differences.

3. I have detected a trend for a behavioural syndrome between activity and risk-taking, more risk-taking animals tended to be more active at the same time. This trend was only apparent in the ‘disturbed group’ further emphasizing the importance of the environment perceived during ontogeny.

4. I have detected various correlations between behavioural types and behavioural precisions in the ‘disturbed group’ of older tadpoles. Complex behavioural strategies were present: highly active individuals were associated with high behavioural predictabilities; shy animals were associated with high unpredictability; and highly predictable explorers were also predictable in their risk-taking. These findings further add to the literature suggesting the

importance of behavioural predictability in behavioural consistency research.

5. I have detected a pace-of-life syndrome in both studied tadpole stages. Both younger and older tadpoles which were more active metamorphosed earlier under the same conditions with *ad libitum* food available. Note that this association was absent in the older ‘disturbed group’. However no POLS were found in the case of exploration and risk-taking behavioural types, and no personality trait correlated with relative mass gain. Multiple correlations with indirect fitness predictors were found in the case of behavioural predictability. Older tadpoles of the ‘naïve group’ had achieved higher relative mass gain when they were more predictable in their activity. Older tadpoles of the ‘disturbed group’ had a higher relative mass gain if the tadpoles were unpredictable in their risk-taking and predictable in their exploration. These findings regarding the pace-of-life syndromes concur with most of the literature: some predictions are detectable and supported, others however are not found. The multiple correlations between fitness predictors and behavioural predictability suggest the possibility of integration into the POLS framework and shows behavioural predictability can be a subject of selection and evolution.

6. The multiple correlations and behavioural patterns, the absence of activity – developmental time till the start of metamorphosis POLS in the ‘disturbed group’ suggest that even standardized behavioural testing can have drastic effects on the personality structure at a later ontogenetic stage. According to these findings current methodologies may have to be reconsidered.

4.2 – The effect of rearing environment and individual experience on the emergence of behavioural consistency

1. I detected that activity had very low repeatability in the ‘control’ and low repeatability in ‘conspecific presence’ treatment groups where in both cases confidence intervals included zero. In treatments with predator presence the repeatability of activity became much higher and significantly different from zero. The repeatabilities observed for risk-taking were lower in all groups, and they all included zero, except for the ‘predator and conspecific’ group. These findings emphasize the role of important environmental stimuli in the formation of

consistent individual differences during ontogeny.

2. I detected between individual correlations in only the 'predator and conspecifics' treatment group, where a strong behavioural syndrome was present meaning that more active individuals were also more risk-taking. Within individual correlations in the form of phenotypic correlations were present in 'conspecific presence' group which cannot be identified as a behavioural syndrome, but it presents a trend of the studied traits to shift in the same direction. These findings suggest that complex patterns of behavioural consistency emerge in the environment with the most complex stimuli.

3. These findings indicate that different levels of behavioural consistency can form independently of each other and may have different adaptive values. Predation is an important stimuli for tadpoles in the creation of consistent individual differences, but behavioural syndromes only form when conspecifics are also present. These finding suggest that complex interactions between the individuals genotype and environment might be the cause here, but in the case of conspecifics presence phenotypic plasticity might also have a strong effect in the formation of behavioural correlations.

5. Conclusions

Behavioural consistency has become a hot research topic in behavioural and evolutionary ecology recently. The existence of consistent personality traits (animal personality), or correlations across functional different behaviours (behavioural syndrome) that constrain individual behavioural repertoire have created the need for explanations of the emergence and widespread occurrence of these phenomena. The 'constraint' hypothesis predicts the existence of genetic, physiological and psychological regulating mechanisms ultimately resulting in consistent individual differences. Many studies have found evidence of these proximate causes or were successful in creating selection lines based on personality. The 'adaptive' hypothesis on the other hand states that behavioural consistency is an adaptive response to the environment. Some studies have found evidence that in wild environments where specific stimuli (for example predation) are present behavioural consistency can be an adaptive response, and it is absent from populations without the presence of these stimuli. Other hypotheses suggest that there is a connection between behavioural consistency and individual states and different strategies are maintained through feedback loops. One

hypothesis integrates behaviour, physiology, cognition and life history into the pace-of-life syndromes (POLS). The POLS hypothesis predicts stable strategies along the slow-fast lifestyle continuum. Most research done in this field had mixed results as most of the predictions were not present at the same time, and some research was even unsupportive.

My results in the field suggest that amphibians are promising models for studying behavioural consistency (also see Wilson and Krause 2012). My research suggest that even minor environmental stimuli can create animal personalities, but relevant stimuli are necessary to create strong personalities and behavioural syndromes. I have shown that predator and conspecific naïve tadpoles have an activity – timing of metamorphosis POLS present at younger and older life stages, suggesting the importance of activity in tadpole life history. Stress stemming from standardized behavioural stress can uncouple the POLS in later life. Further, the observed correlations between the behavioural predictability of activity, exploration, risk-taking and relative mass gain point towards the need for integrating behavioural predictability into the POLS framework. Regarding relevant environmental stimuli the presence of predators seem to be a key in the emergence of consistent individual differences in personality. When predators were present activity was highly repeatable and increase in repeatability for risk-taking was also observed. The presence of conspecifics have only created trends and but when predator and conspecific presence were experienced together behavioural syndromes formed, and conspecific presence alone was enough to create phenotypic correlations. Few studies have investigated the role of phenotypic plasticity in the emergence of behavioural consistency and my results suggest that in the case of agile frog tadpoles complex interactions between genotype and environment are responsible, but phenotypic plasticity might also explain the effect of conspecifics. However more studies are needed to separate these effects and clearly detect the role of environmental stimuli. The important role of individual experience in the emergence of behavioural consistency also draws attention to a serious methodological problem regarding the study of behavioural consistency and suggests that current methodologies based on repeated and invasive testing of individuals will have to be reconsidered.

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