Blood-parasite infection intensity covaries with risk-taking personality in male Carpetan rock lizards (*Iberolacerta cyreni*)

Gergely Horváth, José Martín, Pilar López, László Zsolt Garamszegi, Péter Bertók, Gábor Herczeg

Gergely Horváth ‘corresponding author’; Behavioural Ecology Group, Department of Systematic Zoology and Ecology, Eötvös Loránd University, Pázmány Péter sétány 1/c, H–1117, Budapest, Hungary; gergohorvath@caesar.elte.hu

José Martín; Departmento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutierrez Abascal 2, 28006 Madrid, Spain; jose.martin@mncn.csic.es

Pilar López; Departmento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutierrez Abascal 2, 28006 Madrid, Spain; pilar.lopez@mncn.csic.es

László Zsolt Garamszegi; Department of Evolutionary Ecology, Estación Biológica de Donaña-CSIC, c/ America Vespucio, s/n, 41092, Seville, Spain; laszlo.garamszegi@ebd.csic.es

Péter Bertók; Faculty of Veterinary Sciences, Szent István University, István utca 2, H-1078 Budapest, Hungary; laslegos@gmail.com
running title: Blood-parasites and personality in Carpetan rock lizards

total number of words: 5815
Abstract

Identifying evolutionary and developmental mechanisms underlying consistent between-individual differences in behaviour is the main goal in ‘animal personality studies’. Here, we explored if activity and risk-taking varied consistently between individuals, and correlated to various – potentially fitness-linked – male traits in Carpetan rock lizards (*Iberolacerta cyreni*). Lizards showed significant consistency within both behaviours, implying the presence of activity and risk-taking personalities. However, there were no correlation between activity and risk-taking, neither on the between-, nor on the within-individual levels, implying the absence of a behavioural syndrome. We found a strong link between the intensity of blood-parasite (*Haemogregarinidae*) infection and risk-taking: lizards with higher infection intensity took more risk. While we cannot distinguish cause from causative in the parasite intensity – risk-taking correlation, our results are in line with the asset protection hypothesis predicting that individuals with lower future reproductive value should focus on the current reproductive event and take higher risk.

Keywords: animal personality, behavioural syndrome, behavioural type, individual quality, lizard, temperament
Introduction

Behavioural consistency within (animal personality) and across (behavioural syndrome) different behaviours have become well-studied during the last decade and they proved rather common among a wide range of animal taxa (Smith & Blumstein 2008; Garamszegi et al. 2012). In general, behavioural consistency limits individual behavioural variation including adaptive plasticity, which seems intuitively maladaptive (Sih et al. 2004a, b, 2012; Bell 2005, 2007; Sih & Bell 2008; Sih & Del Guidence 2012). Since behaviour is one of the most plastic quantitative traits of animals (West-Eberhard 2003), the most important question emerging is: why behavioural consistency exist in the first place? The importance of genetic and physiological constraints (proximal theory) alongside adaptive mechanisms (ultimate theory) are well cited in personality research (Koolhaas et al. 2007, Sih & Bell 2008). Recently, several studies aimed to establishing new conceptual frameworks based on life-history differences or combining proximate and ultimate mechanisms viewing behavioural personality from an adaptive perspective (see Pace of Life Syndromes, Réale at al. 2010 and behaviour-state feedbacks, Sih & Bell 2008; Dingemanse & Wolf, 2010; Sih et al. 2015).

Besides of adaptive explanations for the existence of behavioural consistency, many scholars draw attention to the important role of ecological factors shaping behavioural variation within populations (Bell & Sih 2007; Cote et al. 2008; Sih et al. 2015).

One crucial step towards understanding the mechanisms behind behavioural variation in the wild is to link behaviour to individual quality, however, this is not straightforward in most cases, mainly because of the controversy around the definition and measurement of ‘individual quality’. Since individual quality can only be approached through fitness, a viable and relatively easy solution to this problem can be focussing on individual traits that are
linked to fitness (Wilson & Nussey 2010; Bergeron et al. 2011; Bajer et al. 2015). Importance of parasitism in changing behaviour of hosts has long been known and their role in the evolution of animal personalities and behavioural syndromes as potential selective agents gained much attention lately (Barber & Dingemanse 2010, Kortet et al. 2010; Hammond-Tooke et al. 2012; Poulin 2013; García-Longoria et al. 2014). For instance, parasites can act directly creating behavioural differences within and between populations (e.g. reducing exposure to parasites), affecting the emergence of behavioural consistency (Barber & Dingemanse 2010). Alternatively, since infection impacts hosts’ condition, energy stores, reproductive value and vitality in a negative way, parasitism can trigger behavioural changes indirectly by altering the state of an individual (Barber & Dingemanse 2010; Kortet et al. 2010; Sih et al. 2015). Hence, using parasite infection-rate as a fitness proxy gives us the possibility to establish relationships between fitness and individual behaviour, ultimately leading to hypotheses about the behaviour – individual quality link we were looking for.

Negative effects of blood parasite infection on fitness are known from many reptile species (Amo et al. 2004, 2005; García-Ramírez et al. 2005; Garrido et al. 2014, Molnár et al. 2013). In Carpetan rock lizards (*Iberolacerta cyreni*), it is known that parasite load is an indicator of individual quality since the rate of blood-parasite (*Haemogregarinidae*) infection was found to be positively correlated with the body condition in the males of this species (Amo et al. 2004). Besides of this, *I. cyreni* has become one of the most studied reptiles in the past 15 years and it is known that several individual traits of males play an important role in both intra- and intersexual selection of the species. For instance, number and asymmetry of femoral pores of male Carpetan rock lizards provide information about the individuals’ genetic quality, fighting ability and condition (Martín & López 2000, 2006; López et al. 2002, 2003, 2006). In addition, head and body size are both known to affect dominance in
reptiles, moreover, the length of limbs is also a potential fitness proxy due to its link to sprint speed (Bauwens et al. 1995; Gvozdik & Van Damme 2003; Roff 1992). Therefore, *I. cyreni* provides an excellent model to link behaviour to individual quality. Further, behavioural consistency research on reptiles is negligible compared to mammals, birds, or fishes (Smith & Blumstein 2008; Garamszegi et al. 2012, 2013), thus using a lizard as model can be beneficial to broaden the taxonomical sample in this area.

In this paper, we studied whether (i) wild-caught male *I. cyreni* during the mating season showed behavioural consistency and, assuming that individual behavioural variation is present, (ii) individual behaviour covaried with blood parasite load and other potentially fitness-related traits. To this end, we performed behavioural experiments under semi-natural conditions to assess activity and risk-taking of reproductive, adult male lizards. We followed a repeated-measure design in which we assayed each individual 5-6 times over a 13 day period. Since haemogregarines has negative, but not directly lethal effects on their lizard hosts (Oppliger et al. 1996; Veiga et al. 1998; O’Dwyer et al. 2004; Bouma et al. 2007) parasite – behavioural links are expected (Barber & Dingemanse 2010, Kortet et al. 2010). However, considering the correlative nature of our study, separating cause and effect in our findings is not straightforward. For instance, individuals with higher behavioural activity may encounter parasites more frequently (García-Longoria et al. 2014) or individuals with higher parasite load and thus lower future reproductive value might take more risks following the asset protection principle (Clark 1994). Besides the intensity of blood-parasite infection, we also included a number of other potentially fitness-linked traits in our study to seek for other signs of behaviour – individual quality links.

**Methods**
Study animals

We noosed adult males (N = 24) with intact or well regenerated tails during the mating season (early June, 2013) at ‘Puerto de Navacerrada’ pass (Sierra de Guadarrama, Madrid Prov., Spain, 1900 m asl approximately). During this season this habitat has relatively dense vegetation (e.g. shrubs and grass meadows) and there is also high cover of granite rock-boulders (Martín & Salvador 1997; Martín & López 2013). Before housing animals, we measured various morphological and colour traits and took blood samples to quantify the level of blood-parasite infection. The males were housed outdoors individually in grey, opaque boxes (56.5 cm × 36.5 cm × 31.4 cm; length, width, height, respectively) at ’El Ventorrillo’ field station, 5 km from the capture site. In the boxes we used a layer of coconut fibres as substrate and hollow bricks were provided as shelters. Shelters were removed from the boxes every morning and replaced every evening to provide night-refuge but also to avoid the problems imposed by individuals being in shelter during movement activity assays. This procedure never cased any visible stress lasting longer than a few seconds. Between assays, the boxes were covered with a fine metal mesh to protect the lizards from bird predators. Before the onset of experiment, the animals were allowed to habituate to their new environment for 10 days. During this time, and later during the experiment, water and food (house crickets, *Acheta domestica*; Turkestan cockroaches, *Blatta lateralis*) were provided every afternoon (after 14.00 pm (UTC + 02.00), when the behavioural assays ended) *ad libitum*. We note that uneaten food was removed at the evening, so that food was only available for ca. 5 hours in excess every afternoon. Hence, lizards during the time of the behavioural assays (see below) were already foraging. At the end of the experiment, lizards were released at their original capture point.
Individual traits were measured at the ‘El Ventorrillo’ Field Station before transporting individuals to their home cages. Snout-vent length (SVL), head size (represented by head-length, head-width, jaw-width and head-height) and the length of the limbs were measured using a digital calliper to the nearest 0.01 mm. To characterise head size, we ran Principal Component Analysis on the head measures. We got a single principal component with strong positive loadings (proportion of variation explained = 66%; factor loadings > 0.67) and we used it as our head size variable. To characterise limb length, we measured the left and right femurs and tibias, and then summed the mean femur and tibia lengths for every individual. Due to the length of the experiment during which the animals received food ad libitum, we did not analyse body weight. Note that adding body weight measured at various stages during the experiment to our models never changed the results qualitatively (data not shown).

We used 25 G insulin syringes to take blood from the individuals. Blood was drawn from a large subcutaneous vessel on the ventral side of the animals to investigate the rate of blood-parasite (haemogregarine) infection. This method caused no permanent injuries and the animals showed no sign of abnormal behaviour after the procedure. Blood was collected using 60 µl haematocrit capillary tubes (VWR International Ltd., Debrecen, Hungary) and blood smears were made by blowing a drop of blood onto the microscope slide. Smears were air-dried until coagulation. Fixation, staining of blood-smears and estimation of parasite intensity (corrected for red blood cell density) were done following the method of Molnár et al. (2013).

Behavioural assays
Activity and risk-taking of lizards were tested between 13 and 25 June, 2013 on sunny, low wind days. Observations took place in every second day to leave animals time to recover from the handling related to the risk-taking assay (we skipped an observation on 19 June because of thick cloud cover and light rain).

Activity of animals was measured five times (i.e. in five different days) in their home boxes. Video footages were made of the lizards’ movements between 11.15 am and 12.15 pm (UTC + 02.00) using webcams (LifeCam HD-3000, Microsoft Co., Redmond, Washington, USA) and digital video cameras (Panasonic HC-V100, Panasonic Co., Kadoma, Osaka, Japan). We analysed the movements of the animals using the programme MATLAB (MathWorks, Natick, Massachusetts, USA). We used 30 minutes of every video footage (sum of three 10 minute intervals, distributed equally: 0-10 min., 25-35 min., 50-60 min.) to describe activity of the animals. Total distance moved was used to describe activity (see Réale et al. 2007). We applied a log_{10} transformation to achieve normal distribution.

Risk-taking was tested six times (i.e. in six different days) during afternoons (between 12.30 pm and 14.00 pm; UTC + 02.00) in unfamiliar, hence, potentially risky environments. These tests were run on the same days as the noninvasive activity assays (average time interval between activity and risk-taking assays was 15 minutes). However, due to camera malfunction, we had a day when only risk-taking could be measured. Eleven arenas (black plastic boxes, 36.5 cm × 22.4 cm × 25 cm, length, width, height, respectively) were used for the risk-taking tests. A smaller white opaque box (starter-box; 11.2 cm × 7.3 cm × 5.4 cm; length, width, height, respectively) with a manually removable cardboard door was placed in each arena. First, the test animals were caught and placed into the starter boxes, and we let
them acclimate for five minutes. Then, we removed the door and video recorded (Panasonic HC-V100, Panasonic Co., Kadoma, Osaka, Japan) the animals’ behaviour for 10 min. Time till the lizards left their refuges (when the lizards’ midbody [from head to the cloaca region] emerged from the shelter) was used as a proxy for risk-taking with individuals emerging quickly seen as risk-takers (Cote et al 2010, Beckmann & Biro 2013). Tests were performed in three turns on each day, to assay all lizards once in every test day. The order and placement of the animals were randomised within day. The risk-taking arenas and starter-boxes were washed thoroughly with detergent between tests to remove chemical stimuli that may have left by the last male.

Individuals that did not leave the refuge after 10 min were treated as expressing an extreme phenotype, being the ‘shiest’ (13 animals, 54 % of all assays), thus we did not exclude them from the analyses to avoid the loss of an important amount of information. On the other hand, giving them the maximal score (600 sec) would mislead us in calculating within-individual variance of risk-taking by arbitrarily decreasing it. Hence, we first rank-transformed the data and gave these individuals a random rank at the end of the distribution (Bajer et al. 2015). Data were then normalized using the Rankit method (Solomon & Sawilowsky 2009).

Statistical analyses

We used the repeated data on each individual as random observations because (i) they had 10 days to acclimate to the settings before the first assay and one day recovery between assays, and thus habituation during the tests should be minimal and (ii) we used a semi-natural setup where the between-day environmental variation could not be controlled. However, to test for potential habituation effects directly, we performed random intercept-random slope linear
mixed models on both activity and risk-taking as response variables, including z-transformed
order of trials as random slopes, individual as random effect and an intercept. We found no
sign of habituation (data not shown).

Bivariate Mixed Models (BMMs) were used to partition variance components at different
levels (Dingemanse & Dochtermann 2013) with the two behaviours as response variables in a
model that contained only an intercept and individual as a random effect. We used the
MCMCglmm R package (Hatfield 2010) that implements a Bayesian framework for model
fitting, and in which we ran 1300000 iterations with 300000 burn-in period, and we sampled
the Markov chain at each 1000th iteration. Within-behaviour consistency was estimated in the
form of repeatability in activity and risk-taking separately. Because phenotypic behavioural
correlations are unreliable in assessing behavioural syndromes, we decomposed phenotypic
correlations between activity and risk-taking into within- and between-individual correlations
(Dingemanse et al. 2012; Dingemanse & Dochtermann 2013; Garamszegi et al. 2013), where
the latter is the true estimator of behavioural syndromes, which, by definition, describes
between-individual variation across behaviours. The results are given as the estimates of
repeatabilities and correlation coefficients and their 95% confidence interval. The model was
run in MCMCglmm using R (R Development Core Team 2010). Since there was no sign of
behavioural syndrome (see Results), we focussed on the individual behaviours in our later
analyses.

Because both activity and risk-taking were repeatable within individuals across assays (see
Results), we could use individual-specific estimates of these behaviours as traits reliably
reflecting some aspects of an individual property. In further analyses, individual behaviour
was represented by behavioural type (the mean of the subsequent measures). Since the two
behaviours were uncorrelated (see Results) we could test the link between behaviour and the
other traits of interest by running separate General Linear Models (GLMs) for activity and
risk-taking. In these GLMs, behavioural types were the response variables, and SVL, relative
head size, relative limb length, number of femoral pores, and intensity of blood-parasite
infection were the predictor variables. We used relative traits (i.e. residuals corrected for
SVL) for the morphological variables in the GLMs instead of raw variables to avoid
multicollinearity. We ran model-diagnostics based on variance inflation factor (VIF) that
indicated no evidence for multicollinearity (all VIFs < 1.24). The GLMs were built without
interactions. We tried backward stepwise model selection, but since the results never changed
qualitatively, we report the original full models. Analyses were performed with R (R
Development Core Team 2010).

Results

Both activity and risk-taking of I. cyreni males were significantly repeatable across 5 or 6
assays, respectively (activity: \( r = 0.69 \), 95% CI range = 0.51 – 0.8; risk-taking: \( r = 0.22 \), 95%
CI range = 0.11 – 0.41). We found no between-individual correlation between the studied
behaviours (\( r = -0.15 \), 95% CI range = -0.61 – 0.24, N = 24), which could have been
suggestive of an activity – risk-taking behavioural syndrome. There was no sign of within-
individual correlation either (\( r = 0.01 \), 95 % CI range = -0.2 – 0.19, N = 24).

None of the predictor variables were related to activity (Table 1). However, there was a strong
correlation between risk-taking and blood-parasite intensity, lizards with higher infection rate
taking higher risk (\( t = -3.3, P = 0.004, r = -0.61 \), 95% CI = -0.79 – -0.23, N = 24) (Fig. 1). The
effect size of this relationship can be interpreted to represent a large effect (Cohen, 1988).

None of the other variables affected risk-taking (Table 1).

Discussion

The most prominent outcomes of the present work are two-fold. First, adult male *I. cyreni* showed significant activity and risk-taking personality over the observed period. Second, risk-taking was strongly and positively correlated to the intensity of haemogregarine infection across the studied individuals. We did not find any sign of a between-individual correlation regarding activity and risk-taking, hence, the presence of a behavioural syndrome was not supported.

We found that activity and risk-taking are both repeatable within-individuals. The repeatability of behaviour is generally around 0.3-0.4 (Bell et al. 2009). Hence, the behavioural consistency of *I. cyreni*’s activity (*r* = 0.68) is high, while the consistency of risk-taking was rather low (*r* = 0.22). This adds to the slowly accumulating literature of reptilian behavioural consistency, where the major personality axes (*sensu* Réale et al. 2007) are usually found to represent personality (Cote and Clobert 2007; Cote et al. 2008; Rodríguez-Prieto et al. 2011, Carter et al. 2012a, b; Le Galliard et al. 2013, 2015; McEvoy et al. 2013, 2015; Highcock & Carter 2014; Teyssier et al. 2014, Bajer et al. 2015, Kuo et al. 2015).

However, despite the support for behavioural consistency within behaviours, we found no evidence for behavioural consistency across behaviours as indicated by the low and nonsignificant between-individual correlation. This incongruence between the two levels of behavioural consistency strengthen the notion that behavioural consistencies within and between functionally different behaviours (animal personality and behavioural syndrome,
respectively) are different phenomena and should be treated as such (Garamszegi & Herczeg 2012; Jandt et al. 2014).

Males with high blood-parasite (haemogregarine) infection took more risk than their healthier conspecifics. These parasites have been found in fishes, birds, amphibians and reptiles (Smith et al. 1994; Caudell et al. 2002; Lainson et al. 2003), including other lacertids (see e.g. Molnár et al. 2013; Garrido et al. 2014;). Haemogregarine infection has mild effects on the hosts, however, it does depress haematocrit levels which impacts (by the reduced haemoglobin concentration and insufficient oxygen supply of muscles) several physiological and behavioural traits (e.g. Veiga et al. 1998; O’Dwyer et al. 2004). In general, parasitism can be linked to risk-taking behaviour via numerous causal mechanisms (Kortet et al. 2010; Poulin 2013). For example, as an outcome of high parasite-infection, individuals with low assets (low probability of survival till the next reproductive event) might apply higher risk-taking strategies in a current breeding situation than healthier conspecifics with high assets (asset protection hypothesis, Clark 1994). Further, an active and risk-taking behavioural strategy is expected to result in more contact with parasites and/or in immunosuppression that could lead to higher parasite levels in risk-taker individuals. However, parasites with strong negative effects on their hosts’ health are expected to lower behavioural activity. Hence, higher risk-taking might be coupled with higher infection intensity with parasites with mild effects, a hypothesis that was supported by a recent comparative study using 44 birds species (García-Longoria et al. 2014) and is also in line with our results. It is known for both I. cyreni and Lacerta viridis that haemogregarine infection intensity correlates positively with male body condition during the reproductive season (Amo et al. 2004; Molnár et al. 2013), probably as a result of the higher testosterone levels of males in good body condition (Amo et al. 2004). Since lizards are known to act as definitive hosts for haemogregarines, adaptive manipulation
of the host behaviour seems unlikely, however, parasites have the potential to alter the host’s
personality in a multidimensional way through pathological effects (Hammond-Tooke et al.
2012; Poulin 2013). Activity was highly repeatable, representing a strong personality trait in
our population. However, none of the predictors tested in this study were correlated with
activity. Movement activity in a familiar environment might have strong physiological control
unrelated to the measured individual characteristics, and be linked to life-history variation
(Réale et al. 2010). Future studies should involve the relevant physiological and life-history
traits as predictors.

Taken together, I. cyreni males have activity and risk-taking personalities during the mating
season. We note that while the ca. two weeks period of our assays is insufficient for drawing
conclusions about personalities lasting through the year or life, we are confident that the
reported behavioural consistency is present during the relatively short and synchronised
breeding season in our species. We did not find any trend indicating the presence of a
behavioural syndrome, suggesting that animal personality and behavioural syndrome are not
tightly linked phenomena. Finally, we found a strong positive correlation between blood-
parasite infection intensity and risk-taking. This is in line with the predictions of the asset
protection hypothesis. However, since our study is entirely correlative, it is also possible that
risk-taker individuals simply have higher chance to be parasitized than risk-averse
individuals. We note that our results seem to be somewhat incongruent with previous results
reported by López et al. (2005), who found that healthier lizards with better body condition
took higher risk. However, they assessed risk-taking in the lizards’ own natural home ranges,
while we assessed risk-taking in a novel area. The different situations might result in different
costs and benefits for leaving a refuge after a simulated predatory attack. Future manipulative
experiments are necessary for a better understanding of risk-taking variation in this
evolutionary ecology model species.

Acknowledgements

We thank Boglárka Mészáros for her help construct the experiment. In addition our sincere
thank goes to Renáta Kopena for her assistance in collection I. cyreni males.

We thank ‘El Ventorrillo’ MNCN Field Station for use of their facilities. Captures and
observations of lizards were performed under license (permit number: 10/024398.9/13) from
the Environmental Agency of Madrid Government (“Consejería de Medio Ambiente de la
Comunidad de Madrid”, Spain). Our work was funded by the Hungarian Scientific Research
Fund (# OTKA-K 105517) and Gábor Herczeg was also funded by the János Bólyai Research
Scholarship of the Hungarian Academy of Sciences. László Zsolt Garamszegi received funds
from the Spanish government within the frame of the “Plan Nacional” programme (ref. no.
CGL2012-38262 and CGL2012-40026-C02-01). Pilar López and José Martín were supported
by the project MICIIN-CGL2011-24150/BOS.

Literature Cited

Amo, L., López, P., Martín, J. 2004: Prevalence and intensity of haemogregarinid blood

Amo, L. López, P., Martín, J. 2005: Prevalence and intensity of haemogregarine blood
parasites and their mite vectors in the common wall lizard, Podarcis muralis. Parasitol Res


Smith T.G., Desser S.S., Martin D.S. 1994: The development of Hepatozoon sipedon n. sp. (Apicomplexa: Adeleina: Hepatozoidae) in its natural host, the Northern water snake (Nerodia sipedon sipedon), the culicine vectors, Culex pipiens and Culex territans, and an intermediate host, Northern leopard frog (Rana pipiens). Parasitol Res 80,559–568
Sih, A., Mathot, K.J., Moirón, M., Montiglio, P.O., Wolf, M., Dingemanse, N.J. 2015:


Figure 1. Correlation between risk-taking and intensity of parasite infection in adult male Iberolacerta cyreni. Intensity of parasite infection is represented by the number of haemogregarinae parasites corrected for red cell count. Risk-taking is represented by latency to leave refuge, hence, small values translate to high risk-taking. Both variables are normalized.
Table 1. Results of the General Linear Models. t statistics (numerator df = 1; denominator df = 23) and their significances (in parentheses) are shown. Significant effects are in bold font.

<table>
<thead>
<tr>
<th></th>
<th>Activity</th>
<th>Risk-taking</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVL</td>
<td>-0.62(0.55)</td>
<td>-0.45 (0.66)</td>
</tr>
<tr>
<td>Relative head size</td>
<td>-0.52 (0.61)</td>
<td>-0.32 (0.75)</td>
</tr>
<tr>
<td>Relative size of limbs</td>
<td>1.21 (0.24)</td>
<td>-0.42 (0.68)</td>
</tr>
<tr>
<td>Number of femoral pores</td>
<td>0.6 (0.56)</td>
<td>-1.42 (0.17)</td>
</tr>
<tr>
<td>Parasite intensity</td>
<td>0.77 (0.45)</td>
<td><strong>-3.3 (0.004)</strong></td>
</tr>
</tbody>
</table>