Metamorphosis reverses the risk-taking behavioral phenotype in moor frog along a latitudinal gradient

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ABSTRACT

Understanding how demographic processes and environmental conditions affect behavioral variation across populations is pivotal in evolutionary biology. However, as strong evidence accumulates for how these factors drive differences in behavior across populations, the role that such processes play in the link between behavior and life-history traits remains unclear. To study this, we collected moor frog eggs (*Rana arvalis*) from multiple populations ranging along a 1700 km latitudinal gradient in Northern Europe. We then raised tadpoles in a common garden experiment, assessing developmental growth and risk-taking behavior of over 300 individuals when reaching well-known larval developmental stages in anurans (tadpoles, Gosner stage 32, and froglets, Gosner stage 42). We found striking behavioral differences across regions and developmental stages. Specifically, tadpoles raised from northernmost regions took shorter times to leave a shelter, were more active and more exploratory in an open field test than tadpoles from southern populations. These behavioral patterns were reversed when individuals were evaluated as froglets in comparisons between these regions. Further analyses incorporating life-history traits indicated a strong association between developmental growth and time to leave the shelter, with contrasting patterns across developmental stages. Together, our results reflect a decoupling of correlations between behavioral and life history traits across developmental stages in species with complex life cycles as a response to the ecological and demographic constraints that they endure.

Keywords: amphibian**,** dual post-glacial recolonization route, POLS, pace-of-life

INTRODUCTION

Behavioral variation among and within animal populations is widespread, yet the mechanisms driving the underlying variation is a subject of great interest (Wolf and Weissing, 2012). Animal behavior is less flexible than traditionally thought and there is compelling evidence that differences in behavior between individuals are consistently heritable (Bakker, 1986; Bouchard and Loehlin, 2001; Groothuis and Carere, 2005; Wilson *et al*, 1994). Consistency in behavior across time is common within animal populations and can constrain individuals' responses to environmental variation, ecology, and predation. In parallel, natural selection is an important driver of behavioral variation across populations (Clark and Ehlinger, 1987; Machado, 1992). For instance, local selective pressures acting on life history traits such as body mass or growth, might induce adaptive processes with important consequences for understanding spatial patterns of behavioral evolution (Culumber, 2022; Dammhahn et al., 2018). However, other evolutionary processes such as demographic processes (e.g. post-glacial colonization) have been far unexplored in relation to life history traits and its correlation to behavioral variation over broad latitudinal scales.

Latitudinal gradients are one of the most important biogeographic patterns on Earth (Fischer, 1960). Species distributions over extensive latitudinal gradients offer a unique opportunity to combine the study of neutral and adaptive genetic variation (Cortázar-Chinarro *et al*, 2017; Rödin-Mörch *et al*, 2019) with behavioral variation across populations of the same species (Golab *et al*, 2020; Samia *et al*, 2017). To date, most studies assessing these questions have focused on how behavioral traits change when animals from different populations across latitudinal gradients interact with its environment (Cassidy *et al*, 2020; Foster *et al*, 2015; Gerlai and Csányi, 1990; Higgins *et al*, 2022; Mitchell *et al*, 1977). However, open questions remain on how behavioral variation is linked to different developmental stages across population's at large latitudinal scales.

Amphibians exhibit complex life cycles (CLC) that include an abrupt change, termed metamorphosis, in individual morphology, physiology and behavior when the aquatic larvae become terrestrial juveniles (Cohen, 1985; Wilbur, 1980). Consequently, amphibian larvae and more terrestrial juveniles vary greatly in many ecological aspects and occupy very different trophic niches (Kelleher *et al*, 2018; Rowe and Ludwig, 1991; Wilbur, 1980). Most anuran larvae are herbivores or omnivores, while metamorphosed individuals are semi-aquatic carnivores and their dispersal and reproduction occur at this stage (Wilson and Krause, 2012). Understanding differences in behavior between developmental stages in populations differing in important life history traits like growth and developmental rates is crucial for behavioral ecology but much understudied to date (but see Brodin et al. 2013).

North European populations of moor frog (*Rana arvalis*) provide a suitable model for investigating evolutionary processes leading to specific behavioral phenotypes and life-history traits. This species has a broad latitudinal distribution with well-documented variation in life history traits across this latitudinal range (Luquet et al. 2019; Räsänen et al. 2008). The species colonized following a dual post-glacial colonization a vast majority of the Scandinavian Peninsula from the north via current Finland and intersected while another lineage that colonized from the South with the contact zone established in central Sweden (Cortázar-Chinarro *et al*, 2017; Knopp and Merilä, 2009; Rödin-Mörch *et al*, 2019). Demographic processes leading to current distribution have been shown to influence such variation. For instance, Luquet et al. (2019) showed that phenotypic variation occurs between the two colonizing lineages along the latitudinal gradient, with strong divergence between the southern and northern colonization routes, with strong selection pressures driving higher growth, development rates and larger body size in the northern regions.

Here, we conducted a common garden experiment with multiple moor frog populations collected within five different regions across northern Europe and performed repeated open field tests on these individuals before and after metamorphosis. Our aim was to assess behavioral variation across populations within the latitudinal gradient at different developmental stages, as well as the consistency of these behaviors across ontogeny. We further combined the study of behavioral traits with the study of life history traits in these individuals to provide novel evidence on the association between behavioral consistency, growth and development across large-scale geographical variation.

We hypothesized that *R. arvalis* will show variation in behavioral traits across the latitudinal gradient because of a combination of selective, environmental and demographic pressures. Shorter breeding seasons due to climate conditions have selected for faster development in high latitude populations of this species (Luquet *et al*, 2019; Meyer-Lucht *et al*, 2019). In concordance, we expect that behaviors associated with risk-taking should have been positively selected in early developmental stages as breeding seasons shorten within the latitudinal gradient, resulting in higher activity, boldness and exploration in high-latitude populations (Laurila et al. 2008). Despite theoretical expectations of selective pressures acting to uncouple behavioral syndromes when environmental conditions experienced across developmental stages differ (Sih *et al*, 2004), a previous study showed consistency in certain risk-taking behaviors across metamorphic stages in anurans (Wilson and Krause 2012). With our study design incorporating measurements of risk-taking behaviors across tadpole and froglet stages and their developmental growth along an extended latitudinal range, we expect to provide further insight on the potential of evolutionary processes to decouple behavioral consistency and its association to life-history traits.

METHODS

Sample Collection and latitudinal gradient

R. arvalis has a broad Eurasian distribution, from the North Sea coast to western Siberia (Sillero *et al*, 2014). We collected eggs of this species in five regions along a 1700 km latitudinal gradient from northern Germany (Hanover) to northern Sweden (Luleå), covering a major part of the latitudinal distribution of the species (Cortázar-Chinarro *et al.*, 2017, Rödin-Mörch *et al.*, 2019). The breeding season along the latitudinal gradient varies from mid-March (Hanover) to late May (Luleå). We collected 50-100 eggs from each of 10 freshly laid clutches (hereafter families) at each of three breeding populations in each region. The distance between populations within the same region varied from 8 to 50 km (average 20 km). However, eggs from only one population were collected in Hanover. The eggs were transported to the laboratory in Uppsala and kept in a climate room at 16°C and 16-hr:8-hr light–dark photoperiod until they hatched and reached Gosner stage 25 (independent feeding; Gosner 1960).

Experimental design

Common garden experiment. We conducted a common garden experiment as described in Luquet et al. (2019) to record the behavior of individuals at two life stages. At Gosner stage 25, 3 tadpoles from each of the 10 families per population were haphazardly selected. These tadpoles were raised individually in 0.75L opaque plastic vials until metamorphosis. They were fed with chopped spinach *ad libitum* and the water was changed every third day. The experiment was conducted in walk-in climate-controlled rooms with 16 °C average water temperature, corresponding to a temperature commonly encountered in the natural environment. The photoperiod was 16L:8D, corresponding to the situation in the middle of the gradient (Uppsala) in May. When tadpoles

reached Gosner stage 32 (Gosner, 1960), we performed the first set of behavioral assays (see below), weighed them and returned them in their vials until they reached metamorphosis (emergence of the first forelimb; stage 42, Gosner, 1960). At metamorphosis, the water was removed and the vials were closed with a lid. When froglets reached stage 46 (tail entirely resorbed), we performed a second set of behavioral assays and weighed them at the end of the assay.

Recording of behavior. We recorded the behavior of tadpoles and froglets individually using an open field test (OFT) in circular arenas (diameter 90 cm) with a circular plastic shelter (diameter 5 cm) in the center in a room at 19 °C. In the tadpole tests, the arenas were filled with 10 cm of water while the arena floor was moist in the froglet tests. We placed individuals in the shelter and left them for 10 min to acclimate. We next opened the shelter and recorded individuals' behavior in the experimental arena with a digital videocassette recorder (Sony, HDR-HC1E Handy Camera) placed 1.70 m above the experimental arena. Using the recordings, we scored emergence time from the shelter, as a proxy for *boldness*, by scoring the time point when the body of the tadpole or froglet had completely emerged from the shelter provided. Emergence from a shelter in an open field test is widely used as a proxy for boldness (Mazué *et al*, 2015). Next, we analyzed the following 5 minutes of the recording to measure another two traits: i) *activity,* obtained as the mean speed during the total recording and ii) *exploration* obtained as the average distance to the shelter during the recorded time. In cases where the individual did not leave the shelter, the trial was stopped after 20 min for tadpoles and 30 min for froglets.

Videos were analyzed using the computer vision software Ctrax (Branson *et al*, 2009) and the quality of the tracking was checked for every individual. When necessary, the tracking was corrected manually using the fixerrors function provided by Ctrax software. We then collapsed the

frame-by-frame positional data obtained and calculated the behavioral proxies using the tracker package (https://github.com/Ax3man/trackr) in R (v4.1.3).

Behavioral traits across the latitudinal gradient

We assessed potential differences in behavior across region of origin and between developmental stages using Linear Mixed Models (LMM) in R (v4.1.3) using lme4 package (Bates et al. 2014, R Core Team, 2017). Each behavior measured was assessed in an independent model. For all models, developmental stage and region were included as a fixed effect, and population of origin and individual identity as random intercept effects. Individual mass at the time of recording was added as a covariate. Full models that provided singular fits were simplified by removing non-informative random effects for each particular model. Significance of fixed effects was tested using a Kenward and Roger modification of F-tests implemented in the lmertest R package (Kuznetsova et al. 2016). Significance of random effects was tested using likelihood ratio test comparing models with or without the tested random effect in the full fixed effect structure. To assess differences between regions, we obtained post-hoc contrasts of the best fitted preference model using the emmeans package and including false discovery rate correction for multiple tests (Lenth 2019). A high number of froglets did not leave the shelter and were assigned to an emergence value of the decided cutoff point (1800 sec; n=146). Thus, boldness data was analyzed using a mixed effect survival test performed with the coxme package in R v. 4.1.3 (R Core Team 2017, Therneau 2018). The model presented the same structure as the LMMs above. Significance of fixed and random effects was tested using likelihood ratio test comparing models with or without the tested random effect in the full fixed effect structure.

Behavioral consistency

We used emergence values obtained in the open field test to measure behavioral consistency of individuals across tadpole and froglet stages. This trait provided the most balanced dataset with information on tests at both stages for 277 individuals. We calculated the repeatability index in a linear mixed model that included log transformed emergence time as the dependent variable, developmental stage, region and its interaction as fixed factors. Population and identity were included as random factors, and mass at the time of recording as covariate. Calculations were performed using the rptR package (Stoffel et al. 2017) in R v.4.1.3. Repeatability index obtained using such methodology is calculated as the relation between the individual variance on the sum of the among and within individual variance (Dingemanse and Dochtermann, 2013; Nakagawa and Schielzeth, 2010). We additionally calculated consistency across developmental stages by means of non-parametric correlation tests (spearman rank), both in the whole dataset and within North and South clusters (see details on genetic clusters below).

To assess behavioral consistency at the individual level we took repeated measures of emergence time in our experimental setup in a subset of individuals (tadpole: n = 30; froglet: n = 33). Individuals used for repeated measurements as tadpoles were not used for these tests as froglets. We evaluated consistency in emergence time at the individual level using two methods: i) calculations of non-parametric correlation tests (Spearman rank) and ii) calculations of repeatability index in a linear mixed model that included region as a fixed factor and population, family and identity as random factors.

Link between behavior and life-history across differentiated genetic regions

We combined data on time to reach metamorphosis (Gosner stage 42, in days) and mass at the same stage to calculate developmental growth (time/mass at Gosner stage 42) for the individuals evaluated for their behavior. Life-history traits for the individuals used in this behavioral study are

a subset of data included in a study on the quantitative differentiation of life-history traits along the latitudinal gradient (Luquet *et al*, 2019; Meyer-Lucht *et al*, 2019). Hence, analyses on differences in life-history traits between regions are omitted in this study and we refer to these studies for more details of their differences across the gradient. Likewise, previous studies on this system found strong genetic differences between northern and southern populations (Cortazar-Chinarro et al. 2017, Rödin-Mörch et al. 2019). Following these findings, in this study we evaluate the link between behavior and life-history across these genetic clusters.

We classified individuals in bold and shy behavioral types using the median value on time to emerge from the shelter. This was done for tadpoles and froglets independently. For tadpoles, the median value to emerge from the shelter was 46 sec (i.e., tadpoles that left the shelter before 46 seconds were classified as bold and over 46 seconds as shy). For froglets the median value to leave the shelter was approximately 30 min (1746 sec), which subsequently divided our dataset in froglets that left the shelter before 30 min (bold) and froglets that did not leave the shelter in 30 min (shy). We focused on this variable to study the link between behavior and life-history since this provided a more complete dataset. Next, we used a LMM with developmental growth as the dependent variable. The model included behavioral type (bold/shy), developmental stage, genetic cluster and their interactions as fixed effects. Population of origin was included as a random factor.

RESULTS

Behavior across regions and developmental stages

Measurements across developmental stages revealed strong effects of developmental stage on boldness (emergence time), activity (median speed) and exploration (distance to shelter) (Figure

1, Table S1). Individuals showed differences in boldness and exploration in relation to the region of origin, but no overall differences in activity (Figure 1, Table S1). Yet, we observed differences in activity across regions driven by the interaction between region of origin and developmental stage (Figure 1, Table S1). A significant interaction between region of origin and developmental stage was likewise observed for boldness and exploration in our study (Figure 1, Table S1)

Independent contrasts of behavioral traits between regions evaluated independently for the two developmental stages showed that in tadpoles, individuals from the northernmost region (Lulea) are bolder than those from the other regions (Figure. 1A; Table S2). Activity in tadpoles decreased from northern regions towards southern regions. However, we found no significant differences in contrasts between the two northernmost regions and in contrasts between the two southernmost regions. In addition, we observed lower activity between Uppsala and the northernmost regions, although our modeling approach established these as non-significant differences after correcting for multiple testing (Figure. 1B, Table S2). We found no differences in exploration across regions at tadpole stage (Fig 1C, Table S2)

Independent contrasts in froglets showed that individuals from the two northernmost regions were significantly less bold (longer time to emerge from the shelter) than individuals from the rest of regions studied in the gradient (Figure 1A, Table S2), Activity of froglets was lowest in the northernmost region (Luleå) and highest in intermediate regions (Uppsala), but no significant differences across regions were found for this behavioral trait (Figure 1B, Table S2). The highest exploration was found in froglets from the southernmost region (Hanover), which significantly differed from all other regions measured in the gradient (Figure. 1C, Table S2). Froglets from the two northernmost regions (Luleå and Umeå) had lower exploration than froglets from southern regions, with the exception of comparisons between Skåne and Umeå (Fig 1C, Table S2).

Behavioral consistency

Analyses of repeated measurements to a subset of 30 individuals during their tadpole stage indicated no consistency in the time to emerge from the shelter at tadpole stage (R = 0 ∓ 0.054; p = 1; Spearman correlation r_s = -0.172, p = 0.362). At froglet stage, we found a significant correlation between the two measurements, although with relatively low repeatability scores (R = 0.292 ∓ 0.128; p = 0.038; r_s = 0.461, p = 0.007).

Repeatability index calculations showed no behavioral consistency in boldness across developmental stages (R = 0.028 ∓ 0.025; p = 0.152). Likewise, we found no consistency across developmental stages in the time that individuals took to emerge from the shelter in correlations that combined data for both genetic clusters (r_s = -0.051, p = 0.400), or when analyzing north and south separately (south: r_s = 0.120, p = 0.187; north r_s = 0.026, p = 0.750).

Association between behavior and life-history

R. arvalis from the northern cluster (Luleå and Umeå) develop at a faster pace than individuals from the southern cluster (Figure 2, Table S3). Overall, individuals with faster times to emerge from a shelter in either developmental stage have faster developmental growth (t = -2.59, df = 620.2, p = 0.010; Table S3). However, this result is driven by a faster development of bolder individuals during tadpole stage as our analyses indicate a significant, but of moderate effect, interaction between boldness and developmental stage (t = 2.05, df = 622.2, p = 0.041). This interaction is driven by a faster developmental growth of individuals classified as bold during tadpole stage, but a slower development of individuals classified as bold during froglet stage (Figure 2).

DISCUSSION

We investigated variation in risk-taking behaviors in *R. arvalis* at two different developmental stages along a 1700 km geographical gradient. We found that at tadpole stage, individuals from high-latitude populations were bolder, i.e. took shorter times to emerge from a shelter, and showed higher activity. This pattern was however reversed as individuals underwent metamorphic changes, with longer times to emerge from a shelter and lower activity in froglets from high-latitude populations. Repeated measurements revealed a lack of consistency in the time to emerge from the shelter within the tadpole stage. Results from froglets showed a significant, but moderate, level of consistency in our behavioral proxy of boldness. Repeated measurements across developmental stages indicate a lack of consistency across the metamorphic boundary in boldness in this species.

We found a strikingly higher risk-taking propensity in *R. arvalis* tadpoles as compared to froglets. These results are likely affected by biological differences between individuals before and after metamorphic changes take place (Wilbur 1980). Moreover, our findings provide novel evidence on the reversal of a boldness proxy across life stages within multiple populations along a latitudinal gradient. We found that individuals from the northernmost region (Luleå) take less time to leave the shelter (~10s on average) at tadpole stage, while metamorphosed froglets from this region leave the shelter later (~1000s on average) than in all the other regions. Our results for tadpoles reflect an increasing pattern in boldness and activity measurements from south to north, and a reversed decreasing pattern in froglets. Such strong differences in a common garden experiment likely reflect genetically encoded responses to environmental cues that these populations endure during development. What factors might be driving the observed reversal in behavioral phenotype along the gradient? It is possible that froglets and tadpoles suffer fluctuating predation pressures due to

amphibian transition from aquatic to terrestrial environments following metamorphosis (Werner 1986, Van Buskirk, 2002; Relyea and Hoverman, 2003, Orizaola and Braña 2005). In tadpoles of other anurans, changes in behavior have been shown to act as an important antipredator response as predator density decreases between latitudes or in between island and continental populations (Laurila et al. 2008, Brodin et al. 2013). Also, susceptibility to parasitic infections have been previously shown as an important factor in behavioral changes across developmental stages in amphibian populations subject to high predation pressures (Szuroczki and Richardson, 2012, Strasburg and Boone 2022). However, we should not expect very intense predation pressures for amphibians in northern European populations studied, as shown for other taxa (e.g. Roslin et al. 2017). While the influence of these factors is plausible, we think, reversal of activity and boldness patterns at metamorphic stages across regions should be a direct consequence of climate-driven differences generating strong time constraints after metamorphosis and prior to hibernation that these populations encounter.

Unlike in our study, previous studies on anurans have found consistency in certain risk-taking behaviors across tadpole and froglet stages. For instance, consistency in activity and exploration before and after metamorphosis has been previously observed in a single northern Germany population of *R. ridibunda* (Wilson and Krause, 2012). It is possible that populations in this region suffer less severe ecological constraints in relation to those from northernmost regions assessed in our study. Indeed, our studied population from a similar latitude (Hanover) presented the lowest drastic changes in risk-taking behaviors across developmental stages (see Figure 1). In addition, Wilson and Krause (2012) found similar results to us in their measured trait that most resemble our boldness proxy (time to leave a shelter when exposed to a predator cue). Consistent differences in boldness and exploration across tadpole and froglet stages have also been observed in *R.*

temporaria populations following isolation by a geographical barrier (Brodin et al. 2013). We suggest that differences with our results might be driven from the strongly differentiated demographic processes leading to population differentiation. A previous study showed that behavioral differentiation between island and mainland populations of *R. temporaria* might be the result of isolation-driven filtering of animal personalities following a founder event (Brodin et al. 2013). The differences observed across our studied latitudinal gradient are similarly driven by a combination of demographic history and local adaptation in response to ecological constraints.

Consistency in activity within the tadpole stage was previously found in *Rana dalmantina* (Urszán *et al*, 2015). We find behavioral consistency in the time to leave the shelter in *Rana arvalis* froglets, but no consistency during tadpole developmental stage. Our results suggest stronger evolutionary pressures selecting for lower intra-individual behavioral variation following metamorphic changes in this species. Unfortunately, our focus on behavioral variation across a large-scale latitudinal gradient and across developmental stages limited our ability to collect large numbers of repeated measures to individuals within the same developmental stage from a single population. As such, we acknowledge that further measurements of within-stage behavioral consistency should be taken before strong conclusions can be drawn on such patterns in relation to latitudinal variables and demographic patterns.

Developmental growth is an important life history trait in larval amphibians, and several studies have shown that this trait readily diverges along climatic gradients (e.g., Berven et al. 1983, Laurila et al. 2008, Orizaola et al. 2010). We found striking differences in the developmental growth between *R. arvalis* populations deriving from the two post-glaciation colonization routes, with the northern clade having higher rate (Luquet et al. 2019; see Figure 2). Further analyses evaluating the association between life history and risk-taking behavior indicate an overall positive

association between boldness and faster growth during tadpole stage, but a negative association following metamorphosis in these same individuals. As with behavioral patterns, the patterns of growth rate-behavior associations observed should be mainly driven by climatic factors. Indeed, season length should be a major factor underlying geographical variation in life history traits in amphibian species (Laurila et al. 2008; Orizaola et al. 2013). Specifically, individuals from populations in climates experiencing shorter periods of adequate temperatures for growth develop at faster rates than those experiencing longer periods of such adequate temperatures (Caruso et al. 2020). Moreover, a prior study across a latitudinal gradient in *R. arvalis* and *R. temporaria* populations has previously shown that northern populations have lower growth rates after metamorphosis suggesting differences in hibernation preparation and in line with our observed behavioral differences (Lyapkov et al. 2009). Overall, the extreme changes in season length across populations studied and the reversal of boldness across developmental stages observed in our common garden experiment suggest that selective pressures imposed by this factor act antagonistically at different developmental stages driving crucial correlations with growth rates across the studied latitudinal gradient. Given the scenario of different selective pressures acting with different strength at tadpole and froglet stages, our data argues for the viability of correlations between behavioral and life history traits to be decoupled across developmental stages.

The pace of life syndrome (POLS) hypothesis predicts a continuum of individual behavioral variation to be associated with life history strategies (Reale et al. 2010). Our observations along the latitudinal gradient seem to fit with predictions at the "fast" end of the continuum in which individuals that are more prone-risk (bolder) also have larger growth rates. This suggests an important adaptive advantage of such strategy during tadpole developmental stages in the species. Yet, as larvae undergo metamorphosis into froglet stage the predictions from the POLS hypothesis

of correlations between boldness and fast growth are no longer observed in our multi-population study. Such findings align well with theoretical and empirical revisitations of the POLS hypothesis incorporating the role of ecological variation and showing variation of these traits across populations exposed to different environmental and ecological conditions (Dammhahn et al. 2018, Montiglio et al. 2018, Polverino et al. 2018, Moschila et al. 2019, Qu et al. 2019). Further study across taxa on how changes across developmental stages drive variation in POLS predictions will be of importance.

In summary, risk-taking behaviors in *Rana arvalis* are clearly differentiated between northern and southern populations studied along a large-scale latitudinal gradient in northern Europe, showing a reversed pattern before and after metamorphosis. Also, natural selective pressure acting on risk-taking behaviors and life history traits seem to act antagonistically across developmental stages in this amphibian species. Together, these results emphasize the importance of latitudinal gradient studies to elucidate evolutionary processes affecting phenotypic variation in behavior among natural populations. To advance our understanding of the causes and consequences of behavioral trait variation, it will be essential to link phenotypic trait variation in behavioral traits between developmental stages with polymorphisms in genomic regions that code for this trait variation to further infer evolutionary processes, historical selection and the maintenance of behavioral trait variation across regions. Furthermore, understanding how demographic and selective processes are linked to geographical changes in animal behavior is an important avenue of future research to advance our ability to conserve species and their habitats in face of the unprecedented climate change.

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Authors contribution

M.C-C: writing original draft, writing-review and editing. A.C-L.: data curation, formal analyses, writing original draft writing-review and editing. D.L.: lab work and editing. E.L.; conceptualization, field work, lab work, writing-review and editing, A.L.; conceptualization, funding, writing-review and editing.

Conflict of interest declaration

We declare we have no competing interests.

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Figure captions

Figure 1. Behavior of moor frogs across the latitudinal gradient. Boldness (A), activity (B) and exploration (C) measured in moor frogs during tadpole (dark green) and froglet (light green) developmental stages. Individuals were brought to the lab from populations that range in a 1700 km latitudinal gradient in Northern Europe and were raised in a common garden experiment. Average values for regions not sharing any letter are significantly different ($p < 0.05$) in post-hoc contrasts of boldness, activity and exploration assessed independently in tadpoles (dark green letters) and in froglets (light green letters; see Table S2).

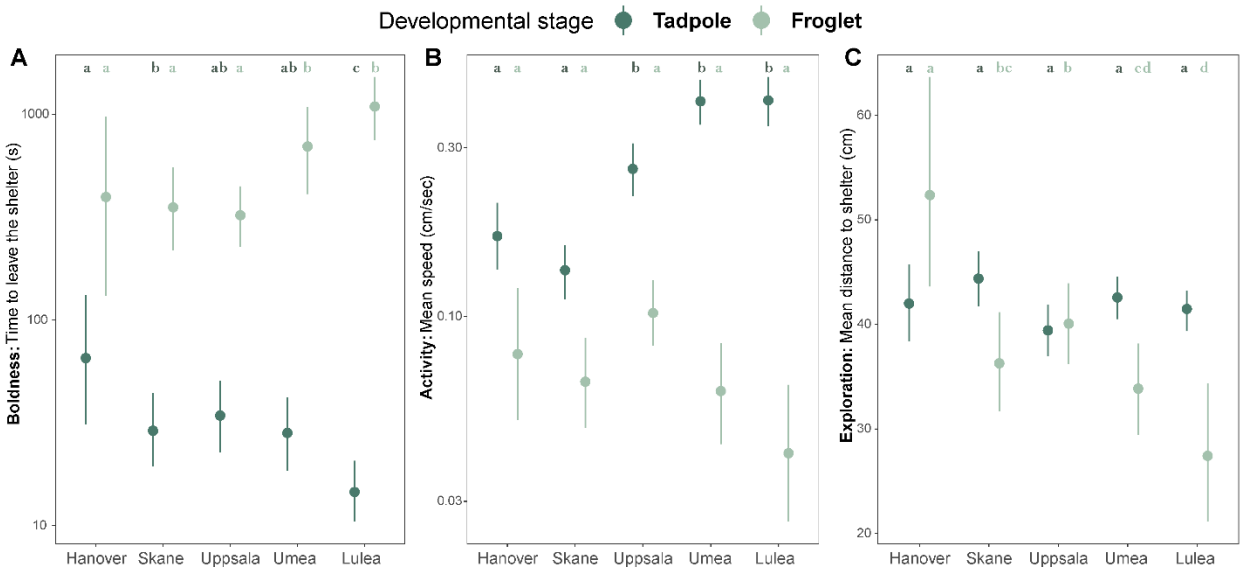


Figure 2. Association between behavioral traits and growth in moor frogs. Genetic cluster of origin had a strong effect on the speed of development. Individuals from Lulea, Umea and Uppsala (North cluster; Blue) developed faster than individuals from Hanover and Skane (South cluster: yellow). Data for both genetic clusters showed that individuals that were classified as bold, scored under median values in time to emerge from a shelter in an open field test, showed faster developmental growth during tadpole stage, but slower developmental growth during froglet stage than shy individuals (see Table S3) .

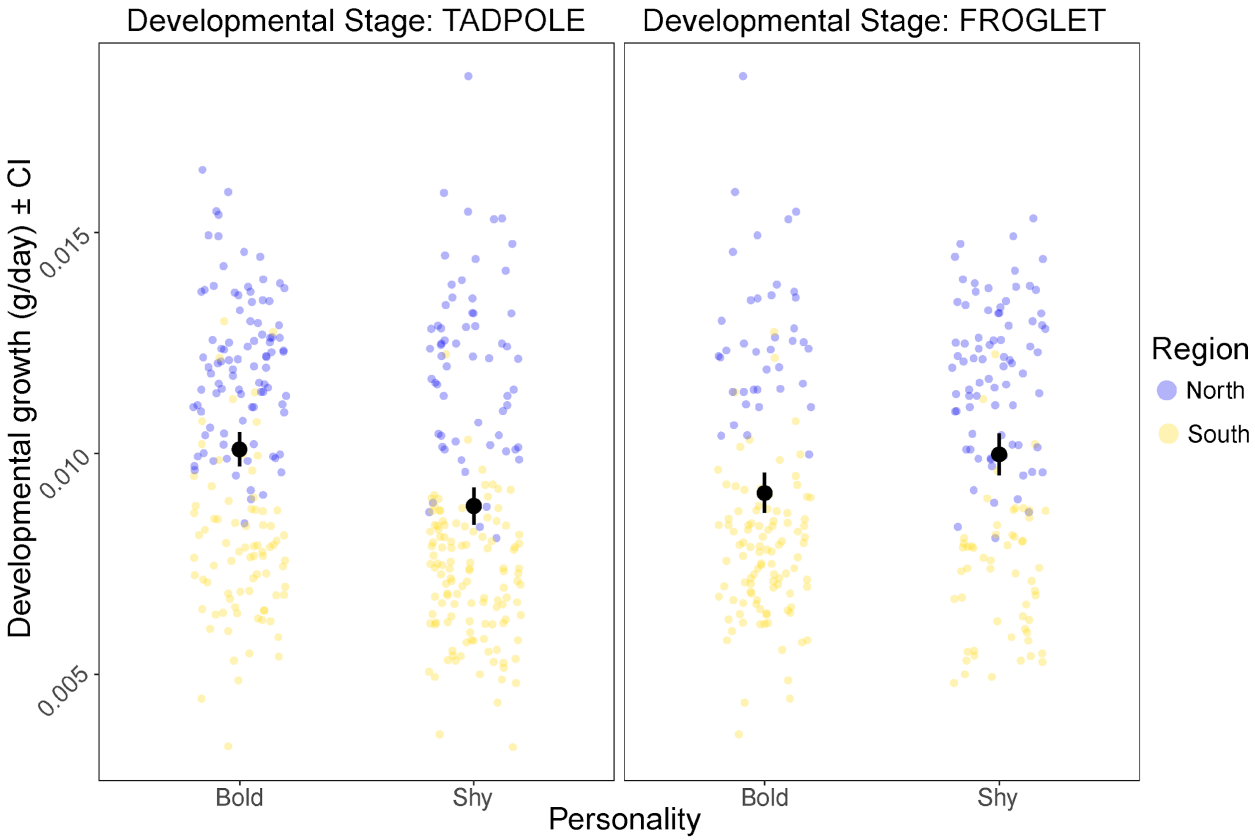


Table S1. Results for statistical analyses evaluating behavioral traits across tadpole and froglet stages in moor frogs originated from 5 different regions across a 1700 latitudinal gradient and raised in a common garden experiment.

Activity: sqrt(speed)					
Predictors	*Sum Sq*	*Mean Sq*	*F value*	*p*	*df(Num:Den)*
Region	0.23	0.05	1.81	0.19	4/10.9
Developmental stage	1.55	1.55	48.67	**<0.001**	1/437.7
Mass at recording	0.12	0.12	3.97	**0.047**	1/500.4
Region * Developmental stage	1.25	0.31	9.81	**<0.001**	4/246.1
Random effects					
σ^2	0.03				
τ_{00}	0.01_{id} $0.00_{population}$				
Observations	512				

Exploration: mean distance to shelter					
Predictors	*Sum Sq*	*Mean Sq*	*F value*	*p*	*df(Num/Den)*
Region	2630.1	657.5	5.35	**<0.001**	4/399.3
Developmental stage	1049.1	1049.0	8.51	**0.003**	1/354.0
Region * Developmental stage	4015.1	1003.7	8.14	**<0.001**	4/337.0
Random effects					
σ^2	123.25				
τ_{00}	16.91_{id} $0.00_{population}$				
Observations	522				

Boldness: Time to leave the shelter					
Predictors	*coef*	*se*	*Chisq*	*p*	*df*
Region	-0.90	0.27	58.57	**<0.001**	8
Developmental stage	1.04	0.31	156.29	**<0.001**	5

Mass at recording	0.87	0.12	4.73	**0.029**	1
Region * Developmental stage	1.25	0.31	48.65	**<0.001**	4
Random effects					
τ_{00}	0.00_{id} $0.05_{population}$				
Observations	655				

Table S2. Independent Contrast between regions for statistical analyses evaluating behavioral traits across tadpole and froglet stages in moor frogs originated from 5 different regions across a 1700 latitudinal gradient and raised in a common garden experiment. Significant p-values adjusted for false discovery rate in bold.

Activity

contrast	*Dev.stage*	*estimate*	*SE*	*df*	*t*	*p*
Hanover - Skane	Tadpole	0.03	0.07	9.33	0.50	0.69
Hanover - Uppsala	Tadpole	-0.11	0.07	9.47	-1.71	0.15
Hanover - Umea	Tadpole	-0.21	0.07	11.01	-3.09	**0.022**
Hanover - Lulea	Tadpole	-0.22	0.07	10.35	-3.24	**0.022**
Skane - Uppsala	Tadpole	-0.15	0.05	9.54	-3.13	**0.022**
Skane - Umea	Tadpole	-0.25	0.05	12.72	-4.85	**0.002**
Skane - Lulea	Tadpole	-0.26	0.05	11.33	-5.16	**0.002**
Uppsala - Umea	Tadpole	-0.10	0.05	11.01	-2.03	0.095
Uppsala - Lulea	Tadpole	-0.11	0.05	10.03	-2.23	0.081
Umea - Lulea	Tadpole	-0.00	0.05	9.50	-0.15	0.87
Hanover - Skane	Froglet	0.01	0.09	29.03	0.13	0.90
Hanover - Uppsala	Froglet	-0.05	0.09	26.55	-0.53	0.78
Hanover - Umea	Froglet	0.04	0.09	32.00	0.40	0.78
Hanover - Lulea	Froglet	0.09	0.10	44.57	0.92	0.73
Skane - Uppsala	Froglet	-0.06	0.06	19.37	-1.02	0.73
Skane - Umea	Froglet	0.03	0.06	29.64	0.40	0.78
Skane - Lulea	Froglet	0.08	0.07	55.29	1.06	0.73
Uppsala - Umea	Froglet	0.08	0.06	22.89	1.40	0.73
Uppsala - Lulea	Froglet	0.14	0.07	47.43	1.92	0.61
Umea - Lulea	Froglet	0.06	0.08	60.42	0.71	0.77

Exploration

contrast	*Dev.stage*	*estimate*	*SE*	*df*	*t.ratio*	*p*
Hanover - Skane	tadpole	-2.40	2.49	12.88	-0.96	0.507
Hanover - Uppsala	tadpole	2.60	2.50	12.95	1.03	0.507
Hanover - Umea	tadpole	-0.62	2.52	13.33	-0.24	0.836
Hanover - Lulea	tadpole	0.52	2.49	12.81	0.21	0.836
Skane - Uppsala	tadpole	5.00	1.78	13.23	2.80	0.146
Skane - Umea	tadpole	1.77	1.80	14.01	0.98	0.507
Skane - Lulea	tadpole	2.92	1.77	12.94	1.65	0.407
Uppsala - Umea	tadpole	-3.22	1.81	14.16	-1.78	0.407
Uppsala - Lulea	tadpole	-2.07	1.77	13.09	-1.16	0.507
Umea - Lulea	tadpole	1.15	1.80	13.86	0.64	0.664
Hanover - Skane	froglet	15.86	4.40	104.87	3.60	**0.001**
Hanover - Uppsala	froglet	12.06	4.25	94.08	2.83	**0.011**
Hanover - Umea	froglet	18.33	4.58	118.61	4.00	**0.001**
Hanover - Lulea	froglet	24.97	5.06	153.78	4.92	**<0.001**
Skane - Uppsala	froglet	-3.79	2.51	48.77	-1.51	0.153
Skane - Umea	froglet	2.47	3.03	93.08	0.81	0.417
Skane - Lulea	froglet	9.11	3.78	161.41	2.44	**0.026**
Uppsala - Umea	froglet	6.27	2.81	72.59	2.22	**0.041**
Uppsala - Lulea	froglet	12.91	3.55	141.14	3.63	**0.001**
Umea - Lulea	froglet	6.638	3.938	186.173	1.686	0.117

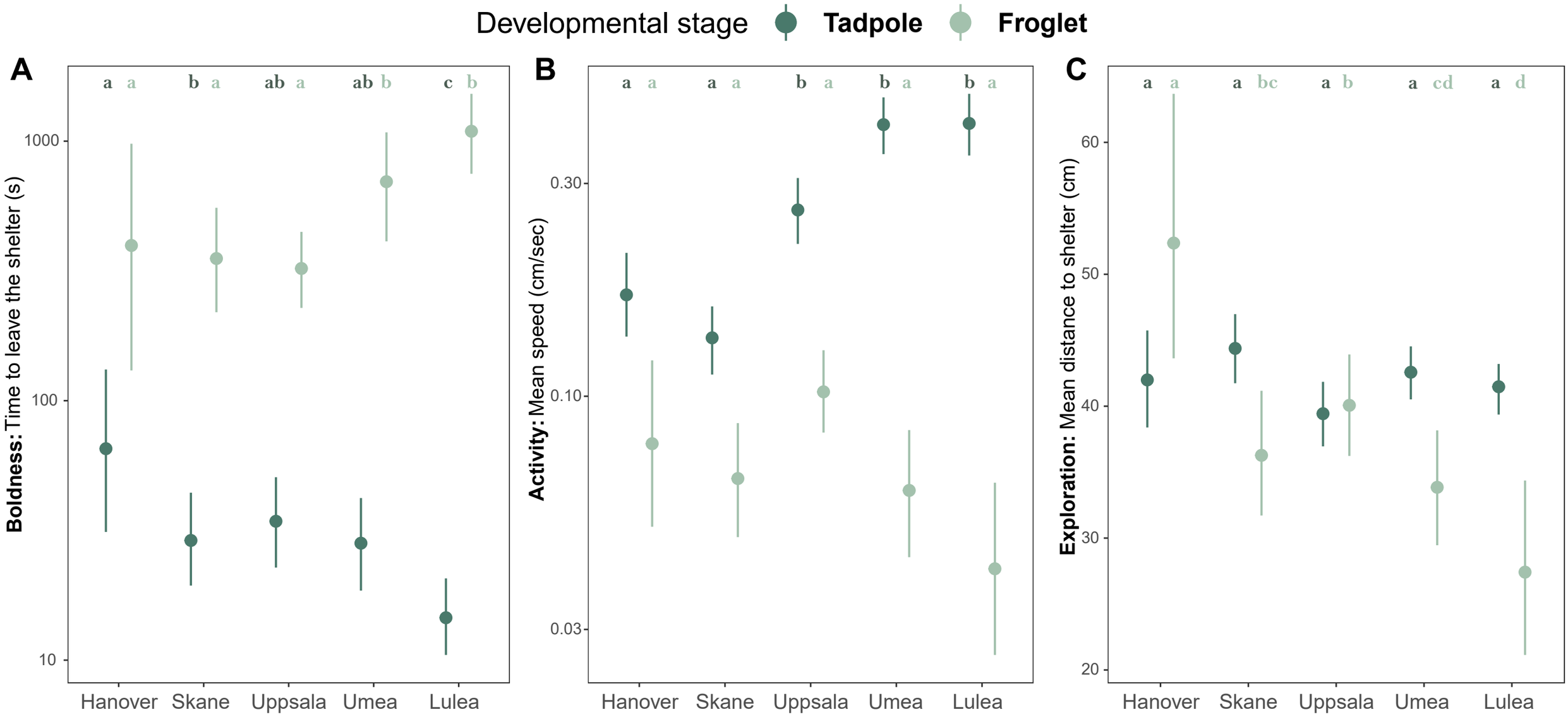
Boldness

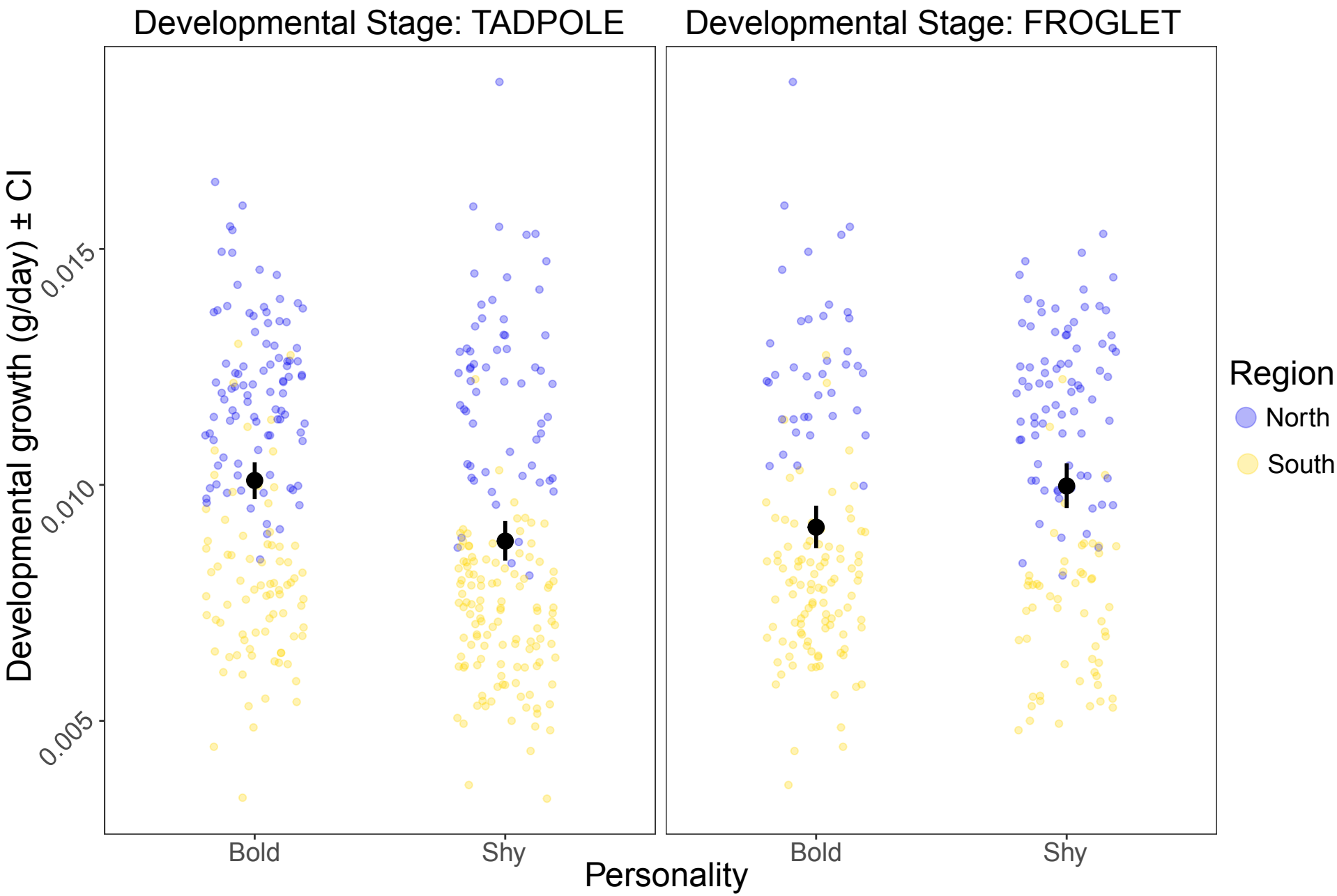
contrast	*Dev.stage*	*ratio*	*SE*	*z.ratio*	*p*
Hanover - Skane	tadpole	0.58	0.13	-2.40	**0.040**
Hanover - Uppsala	tadpole	0.69	0.15	-1.70	0.14
Hanover - Umea	tadpole	0.74	0.18	-1.26	0.29

Hanover - Lulea	tadpole	0.38	0.09	-4.05	**<0.001**
Skane - Uppsala	tadpole	1.17	0.19	0.98	0.36
Skane - Umea	tadpole	1.25	0.25	1.14	0.32
Skane - Lulea	tadpole	0.65	0.12	-2.32	**0.040**
Uppsala - Umea	tadpole	1.07	0.19	0.38	0.70
Uppsala - Lulea	tadpole	0.55	0.09	-3.45	**0.001**
Umea - Lulea	tadpole	0.51	0.08	-4.15	**<0.001**
Hanover - Skane	froglet	1.27	0.34	0.88	0.42
Hanover - Uppsala	froglet	0.98	0.26	-0.07	0.94
Hanover - Umea	froglet	2.01	0.54	2.58	**0.019**
Hanover - Lulea	froglet	2.47	0.67	3.31	**0.002**
Skane - Uppsala	froglet	0.77	0.14	-1.36	0.25
Skane - Umea	froglet	1.59	0.31	2.38	**0.028**
Skane - Lulea	froglet	1.95	0.38	3.40	**0.002**
Uppsala - Umea	froglet	2.05	0.37	3.94	**<0.001**
Uppsala - Lulea	froglet	2.51	0.47	4.91	**<0.001**
Umea - Lulea	froglet	1.22	0.23	1.07	0.35

Table S3. Results for statistical analyses evaluating the relationship between developmental growth and boldness in in moor frogs from 2 genetic clusters (North and South) from a 1700 km latitudinal gradient in Northern Europe and raised in a common garden experiment.

Developmental growth at Gosner stage 42					
Predictors	*Estimates*	*CI*	*t*	*p*	*df*
(Intercept)	0.01259	0.01197 – 0.01321	40.43	**<0.001**	65.4
Personality (*Shy*)	-0.00083	-0.00145 – -0.00020	-2.59	**0.010**	620.2
Cluster (*South*)	-0.00488	-0.00566 – -0.00411	-12.68	**<0.001**	45.4
Dev.stage (*Tadpole*)	-0.00063	-0.00124 – -0.00002	-2.02	**0.043**	620.5
Personality * Cluster	0.00042	-0.00041 – 0.00124	0.99	0.321	619.7
Personality * Dev.stage	0.00086	0.00004 – 0.00168	2.05	**0.041**	622.2
Dev.stage * Cluster	0.00077	-0.00001 – 0.00154	1.94	0.052	618.9
Personality * Dev.stage * Cluster	-0.00110	-0.00219 – -0.00002	-2.00	**0.046**	620.4
Random Effects					
σ^2	0.00				
$\tau_{00\ population}$	0.00				
$N_{population}$	13				
Observations	631				


Developmental stage
Tadpole
Froglet
A
B
C
a a
b a
ab a
ab b
c b
a a
a a
b a
b a
b a
a a
a bc
a b
a cd
a d
Boldness: Time to leave the shelter (s)
1000
100
10
Activity: Mean speed (cm/sec)
0.30
0.10
0.03
Exploration: Mean distance to shelter (cm)
60
50
40
30
20
Hanover
Skane
Uppsala
Umea
Lulea


Developmental Stage: TADPOLE
Developmental Stage: FROGLET
Developmental growth (g/day) ± CI
0.015
0.010
0.005
Bold
Shy
Bold
Shy
Personality
Region
North
South