Metamorphosis reverses the behavioural phenotype in *Rana arvalis* along a latitudinal gradient

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ABSTRACT

1. Understanding how demographic processes and environmental conditions shape behavioural variation across populations is pivotal in evolutionary ecology. However, the role that such processes play in the link between behaviour and life-history traits across populations remains largely unclear.
2. The moor frog (*Rana arvalis*) has colonized Sweden via two distinct routes from south via Denmark and from north via Finland. We collected *R. arvalis* eggs from multiple populations along a 1700 km latitudinal gradient across Northern Europe and raised tadpoles in a common garden experiment. We assessed developmental growth and proactivity levels in ca. 300 individuals at two key stages of anuran larval development: tadpoles (Gosner stage 32) and froglets (Gosner stage 46).
3. We found strong behavioural differences along the latitudinal gradient and between developmental stages. Tadpoles from northernmost populations were bolder (shorter times to leave a shelter) and showed higher activity levels in an open field test compared to those from southern populations.
4. However, these behavioural patterns reversed at the froglet stage, with individuals from northern populations showing reduced proactivity that those from southern populations. Further analyses indicated significant associations between developmental growth and boldness, with contrasting patterns across developmental stages and colonization routes.
5. In line with recent revisitations of the pace-of-life syndrome theory (POLS) for animal personality, our findings suggest a decoupling of correlations between behaviour and life history traits across ontogeny, likely reflecting adaptive responses to divergent ecological and demographic constraints along the latitudinal gradient.

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

INTRODUCTION

Organisms display astonishing variation in behavioural and life history traits (Healy et al., 2019; Stearns, 1992; Wilson et al., 1994; Wolf, 2007). The Pace-Of-Life Syndrome (POLS) has been proposed to explain how the continuum of proactive-reactive behaviours is expected to coevolve with specific physiological and life history traits including metabolic rate, fecundity and growth (Biro and Stamps, 2008; Careau et al., 2008; Réale et al., 2010; Stamps, 2007). In POLS, trade-offs between life history, physiology and behaviour are hypothesized to lead to consistent inter-individual differences in behaviour across time and contexts—often referred to as personality (Bakker, 1986; Bouchard and Loehlin, 2001; Groothuis and Carere, 2005; Wilson et al., 1994). The POLS framework has given valuable insights on how animal personalities may constrain individual responses to environmental variation (e.g. Spiegel et al., 2015, Villegas-Rios et al., 2018) and has been successful in explaining variation especially at species level (e.g. Healy et al., 2019; van de Valle et al., 2023). However, mixed empirical evidence at within-species level suggests a need to integrate additional factors, such as species-specific demographic history and ecological pressures, to better understand the links between behaviour, physiology, and life history (e.g., Chang et al., 2024; Gopal et al., 2023; Montiglio et al., 2018).

Latitudinal gradients represent one of the most important biogeographic patterns on the Earth (Fischer, 1960). Populations at higher latitudes face harsher climatic conditions and shorter breeding seasons - environmental pressures that also influence the composition of predator and

parasite communities and intensity of biotic interactions (Willig et al., 2003; Poulin and Leung, 2011; Schemske et al., 2009). Consequently, environmental conditions at high latitudes drive the evolution of distinct life-history strategies (Stearns 1992). Across large-scale latitudinal gradients, POLS predicts a different pace-of-life along gradients, with populations at higher latitudes showing more proactive, risk-prone behaviours (Cassidy et a.l, 2020; Foster et al., 2015; Gerlai and Csányi, 1990; Higgins et al., 2022; Mitchell et al., 1977). For example, in many anuran tadpoles, more stringent climatic gradient at high altitudes favors faster growth and development rates (Berven et al., 1983; Laugen et al., 2003, Orizaola et al., 2010, Luquet et al., 2019). Faster growth and development rates are linked with higher foraging activity which is likely allowed by lower predator density at high latitudes (Laurila et al., 2006, 2008). Similar relationships have been observed in other environmental contexts (e.g., between island and continental populations: Brodin et al., 2013; along altitudinal gradient: Luquet et al., 2015). However, other studies challenge this pattern indicating a complex relationship between latitude and personality traits. For example, populations of eastern mosquitofish, *Gambusia holbrooki,* show decreased proactivity at high-latitude populations with lower predation pressure (Culumber, 2022). To address these inconsistencies and gain a deeper understanding of how geographical variation influences behaviour and life-history strategies, further research is needed.

Most investigations of POLS focus on individual behaviours at a single life stage (Cabrera et al 2021). However, personality variation may emerge across developmental stages in populations exposed to diverse ecological conditions. This gap should be of particular importance in organisms showing complex life cycles such as amphibians. These species exhibit a dramatic metamorphosis, involving substantial changes in morphology, physiology and behaviour as aquatic larvae transition into terrestrial juveniles (Cohen, 1985; Wilbur, 1980). Consequently, amphibian larvae

89 and juveniles vary greatly in many ecological aspects, for example by occupying distinct trophic
90 niches (Kelleher et al., 2018; Rowe and Ludwig, 1991; Wilbur, 1980). Most anuran larvae are
91 herbivores or omnivores, while post-metamorphic individuals are semi-aquatic carnivores, with
92 dispersal and reproduction occurring at this stage (Wilbur, 1980). The shift of habitat at
93 metamorphosis may also lead to substantial change in predation pressure (Alford, 1999). Under
94 the POLS framework, selection over such differentiated ecological conditions should act to
95 uncouple behavioural syndromes across ontogenetic stages in amphibians (Sih et al., 2004).
96 Previous studies in species with complex life cycles have found both significant consistency
97 (Begue et al., 2024; Brodin 2009; Wilson and Krause 2012) and nonsignificant/weaker
98 relationships (Amat et al., 2018; Brodin et al., 2013; Moncaeu et al., 2017; Plaskonka et al., 2024)
99 in proactivity levels over the metamorphic boundary. This discrepancy between theoretical works
100 and empirical results may result from the lack of consideration of the relationship between
101 personality and life history according to ecological conditions. Consequently, understanding the
102 variation of behaviour in the proactive-reactive continuum across developmental stages in
103 amphibian populations and how they associate to life history across multiple ecological conditions
104 is crucial to resolve this apparent discrepancy.

105 North European populations of the moor frog (*Rana arvalis*) provide a suitable model for
106 investigating evolutionary processes shaping behavioural phenotypes and life-history traits across
107 diverse ecological conditions. This species has a wide latitudinal distribution with well-
108 documented variation in life history traits along this geographical range (e.g., Laurila et al., 2006;
109 Luquet et al., 2019; Räsänen et al., 2008). Following the last glaciation, the species colonized the
110 Scandinavian Peninsula through two distinct routes: one from the north via present-day Finland
111 and another from the south, with a contact zone established in northern central Sweden (Cortazar-

112 Chinarro *et al.* 2017; Knopp and Merilä, 2009,). These post-glacial colonization events and
113 subsequent demographic processes have significantly influenced the divergence between
114 populations. Luquet et al. (2019) demonstrated strong divergence between southern and northern
115 lineages, with strong selection pressures driving higher growth, development rates and larger body
116 size in northern populations. Additionally, genetic analyses revealed clear differentiation between
117 two major genetic clusters corresponding to northern and southern populations (Cortazar-Chinarro
118 *et al.,* 2017; Meyer-Lucht *et al.*, 2019, Rödin-Mörch *et al.*, 2019).

119 In this study, we evaluate behavioural variation across populations, regions and genetic clusters of
120 *R. arvalis* along a latitudinal gradient, incorporating information from two developmental stages.
121 We conducted a common garden experiment using populations from five different regions in
122 northern Europe and performed repeated open field tests on individuals before and after
123 metamorphosis to quantify behavioural traits on the proactive-reactive continuum. This data was
124 combined with life-history measurements to provide insight into the relationship between
125 behaviour, development and life-history across large-scale geographical variation.

126 In *R. arvalis*, shorter breeding seasons and harsher climatic conditions at higher latitudes are
127 associated with faster development (Luquet *et al.*, 2019; Meyer-Lucht *et al.*, 2019). Based on the
128 POLS framework, we hypothesized that i) proactive behaviours would be positively selected in
129 high latitudes at early developmental stages, resulting in a gradient of increasing proactivity-
130 associated traits with increasing latitude; ii) behavioural response on the proactive-reactive
131 continuum would be decoupled across ontogeny due to distinct ecological challenges and selective
132 pressures faced during larval and post-metamorphic stages; and iii) proactive behaviours would
133 positively coevolve with faster developmental growth throughout our geographical range.

134

135 **METHODS**

136 **Sample Collection and latitudinal gradient**

137 *R. arvalis* has a broad Eurasian distribution, from the North Sea coast to western Siberia (Sillero
138 et al., 2014). We collected 50-100 eggs from each of 10 freshly laid clutches (hereafter families)
139 at each of three breeding populations within five regions along a 1700 km latitudinal gradient from
140 northern Germany (Hanover) to northern Sweden (Luleå), covering a major part of the latitudinal
141 distribution of the species (more details about field sampling in Luquet et al., 2019). However,
142 eggs from only one population were collected in Hanover. The timing of breeding season along
143 the latitudinal gradient varies from mid-March (Hanover) to late May (Luleå). The distance
144 between populations within the same region varied from 8 to 50 km (average 20 km). The eggs
145 were transported to the laboratory in Uppsala and kept in a climate room at 16°C and 16L:8D
146 photoperiod until they hatched and reached Gosner stage 25 (independent feeding; Gosner 1960).
147

148 **Experimental design**

149 *Common garden experiment.* Tadpoles were reared in a common garden experiment as described
150 in Luquet *et al.* (2019), however, the individuals included in this study were all reared at the 16 °C
151 temperature treatment. At Gosner stage 25, 3 tadpoles from each of the 10 families per population
152 were haphazardly selected. Tadpoles were raised individually in 0.75 L opaque plastic vials until
153 metamorphosis. They were fed with chopped spinach *ad libitum* and the water was changed every
154 third day. The experiment was conducted in walk-in climate-controlled rooms with 16 °C average
155 water temperature, corresponding to a temperature commonly encountered in the natural
156 environment. The photoperiod was 16L:8D, corresponding to the situation in the middle of the
157 gradient (Uppsala) in May. When tadpoles reached Gosner stage 32 (Gosner, 1960), we performed

158 the first set of behavioural assays at tadpole stage (see below), weighed them to the nearest 0.1 mg
159 and returned them in their vials until they reached metamorphosis (emergence of the first forelimb;
160 stage 42, Gosner, 1960). At metamorphosis, the water was removed and the vials were closed with
161 a lid. On the day when the metamorphs reached the froglet stage (i.e. Gosner stage 46, tail entirely
162 resorbed), we performed a second set of behavioural assays and weighed them to the nearest 0.1
163 mg.

164 *Recording of behaviour.* We recorded the behaviour of tadpoles and froglets (n tadpole / froglet:
165 Hanover = 31 / 20, Skåne = 89 / 65, Uppsala = 90 / 73, Umeå = 89 / 65, Luleå = 90 / 61, total =
166 389 / 284) individually using an open field test in circular arenas (diameter = 90 cm) with a circular
167 plastic shelter (diameter = 5 cm) in the center in a room at 16 °C. In the tadpole tests the arenas
168 were filled with 10 cm of water while in froglet tests the arena floor was moist. Individuals were
169 put in the shelter and left for 10 min to acclimate. The recording started when we opened the
170 shelter. Videos were recorded by a digital videocamera (Sony, HDR-HC1E Handy Camera) placed
171 1.70 m above the experimental arena. Using the recordings, we scored emergence time from the
172 shelter in an open field test, a widely used proxy for *boldness* (Mazué *et al*, 2015), by scoring the
173 time point when the body of the tadpole or froglet had completely emerged from the shelter
174 provided. In cases where the individual did not leave the shelter, the trial was stopped after 20 min
175 for tadpoles (n = 6) and 30 min for froglets (n = 126).

176 For individuals that left the shelter we analyzed 5 minutes of recording to measure proactivity from
177 two additional traits: i) *activity,* obtained as the mean speed during the recording and ii) *exploration*
178 obtained as the average distance to the shelter during the recorded time. Videos were analyzed
179 using the computer vision software Ctrax v0.5.19 (Branson *et al*, 2009) and the quality of the
180 tracking was checked for every individual. When necessary, the tracking was corrected manually

using the ‘fixerrors’ function. We then collapsed the frame-by-frame positional data obtained and calculated the behavioural proxies using the tracker package (https://github.com/Ax3man/trackr) in R v. 4.1.3 (R Core team 2024).

Statistical analysis

Consistency of individual behaviour (personality)

To assess personality, i.e. behavioural consistency at the individual level, we recorded the behaviour of a representative subset of individuals for a second time 24-48 hours later (n tadpole / froglet: Hanover = 2 / 2, Skåne = 7 / 6, Uppsala = 6 / 8, Umeå = 6 / 8, Luleå = 9 / 9, total = 30 / 33). To avoid potential biases introduced by habituation to the experimental setup, individuals that were tested twice as tadpoles were not used for repeated measurements during froglet stage. As we could not obtain repeated measurements of activity or exploration for froglets that did not leave the shelter during the first or second trial (n = 17), we used data on time to leave the shelter to assess the individual repeatability of boldness. Repeatability, the relative partitioning of variance into within-group and between-group sources of variance (Dingemanse and Dochtermann, 2013; Nakagawa and Schielzeth, 2010) was estimated within a linear-mixed model (LMM) framework. We assessed the variance explained by individual identity in boldness (log-transformed emergence time) in independent gaussian models for tadpoles and froglets that included region as fixed effects, and population and individual identity as random factors using the ‘rptR’ package in Rv4.4.1 (Stoffel et al. 2017, R Core team 2024).

Behavioural traits across the latitudinal gradient

203 We assessed potential differences in behaviour across genetic clusters or region of origin and
204 between developmental stages implementing LMMs in 'lme4' package in Rv4.4.1 (Bates et al.
205 2015; R Core Team 2024). Each behaviour measured was assessed in an independent model. For
206 models to assess differences by region, developmental stage, region and their interaction were
207 included as a fixed effect, and population of origin and individual identity as random intercept
208 effects. For models to assess differences by genetic cluster, developmental stage, cluster and their
209 interaction were included as a fixed effect, and population of origin, region and individual identity
210 as random intercept effects. Individual mass at the time of recording was added as a covariate in
211 all models. Model fit and significance were assessed through conditional F-tests with Kenward-
212 Roger approximation for the degrees of freedom using pbkrtest and lmerTest R packages (Halekoh
213 and Højsgaard 2014, Kutznesova et al. 2017). To assess differences between regions, we obtained
214 post-hoc contrasts of the best fitted model using the 'emmeans' package and including false
215 discovery rate correction for multiple tests (Lenth 2018). Significance of random effects was tested
216 using likelihood ratio tests comparing models with or without the random effects in the full fixed
217 effect structure.

218 A high number of froglets did not leave the shelter and were assigned to an emergence value of
219 the decided cutoff point (1800 sec; n=126). Thus, time to emerge from shelter (boldness) data was
220 analyzed using a mixed effect survival test performed with the 'coxme' package (Therneau 2015).
221 The model structure was analogous to the LMMs above. Significance of fixed and random effects
222 was tested using likelihood ratio tests comparing models with or without the tested effect in the
223 full fixed effect structure.

224 We used repeated measurements to same individuals taken during tadpole and froglet stage to
225 estimate the repeatability of behaviours over the metamorphic boundary using analogous LMMs

226 than described above and modelling our data using the 'rptR' package in Rv4.4.1 (Stoffel et al.
227 2017, R Core team 2024).

228

229 ***Link between behaviour and life-history across genetic clusters***

230 We combined data on time to reach metamorphosis (Gosner stage 42, in days) and mass at the
231 same stage to calculate developmental growth (mass/time at Gosner stage 42) for the individuals
232 evaluated for their behaviour. This life-history trait quantified for the individuals used in this
233 behavioural study are a subset of data included in a study on the quantitative differentiation of life-
234 history traits along the latitudinal gradient (Luquet *et al*, 2019; Meyer-Lucht *et al*, 2019). Hence,
235 analyses on differences in life-history traits across the gradient are omitted in this study and we
236 refer to these studies for more details of their differences across the populations sampled.

237 To assess the relationship between behaviour and life-history across genetic clusters and
238 developmental stages, we structured LMMs testing the influence of behavioural traits on growth
239 rate. Behavioural traits (i.e. boldness / activity / exploration, considered independently), genetic
240 cluster or region and developmental stage were fixed effects and population of origin and
241 individual identity were included as random intercept effects. Models fit and significance were
242 assessed as previously through conditional F-tests with Kenward-Roger approximation for the
243 degrees of freedom using pbkrtest and lmerTest R packages (Halekoh and Højsgaard 2014,
244 Kutznesova et al. 2017). We obtained post-hoc contrasts of fitted models using the 'emmeans'
245 package and including false discovery rate correction for multiple tests (Lenth 2018). Significance
246 of random effects was tested using likelihood ratio tests comparing models with or without the
247 random effects in the full fixed effect structure.

248

249 **RESULTS**

250 ***Repeatability of emergence time (boldness)***

251 The time to emerge from the shelter was repeatable (controlling for the effects of region) at froglet
252 stage, but not at tadpole stage (Tadpole: $R = 0.000 \pm 0.060$ *SE*, $P = 1$, Froglet:
253 $R = 0.362 \pm 0.189$ *SE*, $P = 0.023$; Fig 1).

254 ***Behaviour across genetic clusters, regions and developmental stages***

255 *Boldness* – The time to emerge from the shelter was significantly influenced by the interaction
256 between developmental stage and genetic cluster (Table 2). Specifically, boldness did not differ
257 between tadpoles from the South and North genetic clusters, but froglets from South populations
258 were bolder than froglets from North populations (Tadpole $_{\text{North vs South}}$ ratio = 1.26 ± 0.21 *SE,*
259 $z = 1.40$, $P = 0.16$, Froglet $_{\text{North vs South}}$ ratio = 0.50 ± 0.08 *SE,* $z = -4.47$, $P < 0.001$; Fig 2A).
260 Similarly, statistical models incorporating the region of origin indicated a significant interaction
261 effect between developmental stage and region on time to emerge from the shelter (Table 3).
262 Tadpoles from the northernmost region (Luleå) were significantly bolder than those from all the
263 other regions sampled, including those from Umeå within the same genetic cluster (ratio [SE]:
264 Hanover-Luleå: 0.38 [0.09] - *adjP < 0.001*; Skåne-Luleå: 0.65 [0.12] - *adjP = 0.040*; Uppsala-
265 Luleå: 0.55 [0.09] - *adjP = 0.001*; Umeå-Luleå: 0.51 [0.08] - *adjP < 0.001;* Figure. 1D; Table S1).
266 Tadpoles from the southernmost region (Hanover) tended to be shyer (longer time to emerge from
267 the shelter) than those from the other regions although the difference was only significant in
268 comparison to Skåne (ratio [SE]: 0.58 [0.13] - *adjP = 0.040;* Figure. 1D; Table S1). In froglets,
269 individuals from the two northernmost regions were significantly shyer than individuals from the

rest of regions studied in the gradient (Figure 1D, see Table S1). The random population effect was never significant for boldness (Tables 2 and 3 for models incorporating genetic clusters or regions, respectively). Boldness was not repeatable across ontogeny (i.e. between tadpole and froglet stages; $R = 0.079 \pm 0.056$ *SE*, $P = 0.10$; Figure S1).

Activity – Activity was significantly influenced by the interaction between developmental stage and genetic cluster (Table 2) or region (Table 3). At the genetic cluster level, tadpoles from South populations were less active than those from North populations, but there was no difference at froglet developmental stage (Activity: Tadpole $_{\text{North vs South}}$ estimate = 0.18 ± 0.05 *SE,* $t = 3.41$, $P = 0.036$, Froglet $_{\text{North vs South}}$: estimate = -0.06 ± 0.06 *SE,* $t = -0.98$, $P = 0.36$; Fig 2B). At the regional level, there were differences in activity of tadpoles among the regions while in contrast all froglets have similar activity. Specifically for the tadpole stage, the activity increased with latitude: tadpoles from the two southernmost regions (Hanover, Skåne) had a lower activity than tadpoles from intermediate latitude (Uppsala region) and northernmost regions (Umeå, Luleå); the activity of tadpoles from intermediate region (Uppsala) was marginally lower than those from northernmost regions (Fig 2E, see Table S1). The random population effect in activity was significant in genetic cluster model (Table 2) and only marginal in region model (Table 3). Repeated measurements across developmental stages indicated significant, repeatability of activity across ontogeny ($R = 0.158 \pm 0.078$ *SE*, $P = 0.034$; Figure S1).

Exploration – Exploration was significantly influenced by the interaction between developmental stage and genetic cluster (Table 2) or region (Table 3). Exploration of tadpoles from the South and North genetic clusters was similar while froglets from the North genetic cluster showed reduced exploration than those from the Southern cluster (Exploration: Tadpole $_{\text{North vs South}}$ estimate = -1.07 ± 2.09 *SE,* $t = -0.51$, $P = 0.63$, Froglet $_{\text{North vs South}}$: estimate = -9.03 ± 2.76 *SE,* $t = -3.27$, $P = 0.005$;

293 Fig 2C). Specifically, for the froglet stage, the exploration of the southernmost region (Hanover)
294 was higher than all other regions along the gradient (Figure. 2F, Table S14). Froglets from the two
295 northernmost regions (Luleå and Umeå) had significantly lower exploration than froglets from
296 southern regions (except between Skåne and Umeå; Fig 2F, Table S1). The random population
297 effect was never significant (Tables 2 and 3 for models incorporating genetic clusters or regions,
298 respectively). Repeated measurements across developmental stages indicated no repeatability of
299 exploration ($R = 0.099 \pm 0.078$ *SE*, $P = 0.13$; Figure S1).

300

301 ***Influence of behavioural traits on growth rate***

302 *Growth/boldness* - Growth rate was affected by the three-way interaction between boldness,
303 genetic cluster and developmental stage (Fig 3A, Table 4). In the South genetic cluster, tadpoles
304 with fastest times to emerge from the shelter (bolder) showed faster growth rates, but this
305 relationship was not found in tadpoles originating from the North cluster (Figure 3A, Tadpole $_{\text{North}}$
306 $_{\text{vs South}}$ estimate = 0.74 ± 0.28 *SE*, $P = 0.008$,Table S2). This pattern was reversed during froglet
307 stage, as bolder individuals from the North cluster showed faster growth rates as tadpoles, while
308 no association for froglets was found in the South cluster (Figure 3A, Table S2). Similarly,
309 statistical models incorporating the region of origin indicated a significant three-way interaction
310 between boldness, region and developmental stage (Fig S2A, Table 5). This effect was driven by
311 the positive association between growth rate and boldness in Uppsala (estimate $_{\text{Tadpole}}$ = 0.79
312 ± 0.26 *SE*, $P = 0.027$) and Skåne (estimate $_{\text{Tadpole}}$ = 0.89 ± 0.35 *SE*, $P = 0.059$; see Table S3).
313 Additionally, it was driven by i) differential associations of growth rate/boldness associations of
314 these two regions in contrasts with all other regions (see Table S4; Figure S2A); and ii) in contrast
315 with this relationship in froglets in Uppsala (estimate $_{\text{Froglet}}$ = -0.01 ± 0.07 *SE*, Tadpole vs Froglet:

316 t = 2.91, $P = 0.004$; Table S5; FigureS2A) and Skåne (estimate $_{Froglet}$ = 0.11 ± 0.07 *SE*, Tadpole vs
317 Froglet: t = 2.16, $P = 0.031$; Table S5; Figure S2A). The random population effect in activity was
318 significant in models incorporating genetic cluster or region (Tables 4 and 5 respectively).

319 *Growth/activity* - Growth rate was not affected by the three-way interaction between activity,
320 genetic cluster and developmental stage, or by any two-way interactions between these factors
321 (Figure 3B, Table 4). Statistical models incorporating the region of origin indicated a significant
322 three-way interaction between activity, region and developmental stage (Fig S2B, Table 5). This
323 effect was driven by the positive association between growth rate and activity in froglets in
324 Uppsala (estimate = 0.73 ± 0.25 *SE*, $P = 0.032$) and Luleå (estimate = 0.89 ± 0.35 *SE*, $P = 0.059$;
325 see table S3). Additionally, it was driven by i) differential associations of growth rate/activity in
326 Hanover-Uppsala contrasts, as well as contrasts of Hanover, Skåne and Umeå against Luleå
327 froglets (see Table S4; Figure S2A); and ii) in contrast with this relationship when measured as
328 froglets in Uppsala (estimate $_{Froglet}$ = -0.01 ± 0.07 *SE*, Tadpole vs Froglet: t = 2.91, $P = 0.004$; Table
329 S5; FigureS2A) and Luleå (estimate $_{Froglet}$ = -0.01 ± 0.07 *SE*, Tadpole vs Froglet: t = 2.91,
330 $P = 0.004$; Table S5; FigureS2A). The random population effect in activity was significant in
331 models incorporating genetic cluster or region (Tables 4 and 5 respectively).

332 *Growth/exploration* - Growth rate was marginally affected by the three-way interaction between
333 exploration, genetic cluster and developmental stage, and the exploration x developmental stage
334 interaction was significant (Table 4). In the North cluster, there was a negative relationship
335 between exploration and growth rate observed at tadpole, but not at froglet stage (Figure 3C, Table
336 S2). Statistical models incorporating the region of origin indicated an interaction between
337 exploration and developmental stage (Fig S2B, Table 5) driven by a significant change in the
338 direction of this association in Luleå (Tadpole: estimate = -0.15 ± 0.08 *SE*; Froglet: estimate =

339 0.21 ± 0.15 *SE*; t = -2.12, $P = 0.034$; see Table S5; Figure S2A). The random population effect in
340 activity was significant in models incorporating genetic cluster or region (Tables 4 and 5
341 respectively).

342

343 **DISCUSSION**

344 Our investigation of behavioural patterns in *R. arvalis* at two different developmental stages along
345 an extensive geographical gradient highlights the significant variation in proactivity levels across
346 developmental stages, genetic clusters, and regions in this species. We found no individual
347 repeatability of boldness during tadpole stage, but significant repeatability of this trait during
348 froglet stage. Additionally, our analyses of individual repeatability across metamorphosis showed
349 divergent patterns between behaviours quantified, with activity levels showing significant
350 repeatability that was not observed in exploration and boldness. Our results reveal reversals in
351 behaviour across developmental stages in *R. arvalis*, with all traits quantified showing shifts
352 between the tadpole and froglet stages. Finally, growth rate showed complex interactions with
353 boldness, activity, and exploration, varying by genetic cluster, region, and developmental stage.

354

355 **Consistency of inter-individual differences in proactivity and across ontogeny**

356 The repeatability of behaviour was assessed only on the time to emerge from the shelter. The
357 froglets had a repeatable time to emerge from the shelter but not the tadpoles. This suggests that
358 the ontogenetic timing is important to consider in studies investigating the consistency of inter-
359 individual differences in behaviour and assessing personalities. Indeed, studies across taxa support
360 the idea that boldness often develops later in ontogeny, as observed in several vertebrate taxa where

361 boldness may appear only after sexual maturation (Petelle et al. 2013, Polverino et al. 2016, Delval
362 et al. 2020). Moreover, the environmental conditions experienced by previous generations and
363 throughout the lifetime of an individual may also influence the emergence of personalities (e.g.
364 Tariel et al. 2020; Plaskonka et al. 2024). In this study, the results confirm that the time to emerge
365 from a shelter is a proxy of the bold-shy axis of personality in *R. arvalis* and a late emergence of
366 boldness during ontogeny. As it was not the main aim of this study, further assessment of within-
367 stage behavioural consistency is necessary before drawing firm conclusions about when
368 personality emerges and how it is shaped by demographic, geographical and local factors.

369 Repeatability estimates across life stages indicated no consistency in individuals' boldness and
370 explorations over ontogeny while activity was significantly repeatable across tadpole and froglet
371 developmental stages. Previous studies across taxa have reported consistency in individuals'
372 proactivity levels across life stages (reviewed by Koenig and Ousterhout 2018). In anurans,
373 individual consistency in proactivity levels before and after metamorphosis has been observed in
374 the spotted salamander (Koenig and Ousterhout 2018), and in the activity and exploration levels
375 of *Pelophylax ridibundus* from a single population from northern Germany (Wilson and Krause,
376 2012). However, neither Plaskonka et al. (2024) in a single population of Polish *R. arvalis* or
377 Brodin et al. (2013) in several northern Swedish populations of *R. temporaria* found significant
378 repeatability across life stages. Our results are in broad agreement with the previous studies,
379 suggesting that repeatability across life stages can be weak and depend on the environmental
380 constraints. Indeed, our southernmost population exhibited the lowest changes in proactivity traits
381 across life stages, while there was a tendency to gradually increase in change across life stages
382 with increasing latitude (see next parts of discussion). Together, these findings suggest that
383 individual consistency in behaviour across life stages may vary across taxa due to the adaptive

384 value of consistent behavioural responses, shaped to a larger extent by ecological factors than by
385 underlying genetic drivers of behavioural syndromes.

386

387 **Proactive behaviours of tadpoles along the latitudinal gradient**

388 Activity was higher in tadpoles from the North genetic cluster and in the three northernmost
389 regions compared to the South genetic cluster and the two southernmost regions, respectively. In
390 addition, tadpoles from the two northernmost regions also showed a higher activity than the
391 latitudinally intermediate Uppsala region. Consistently, tadpoles from the northernmost region
392 (Luleå) exhibited higher boldness than those from other regions and tadpoles from the most
393 southernmost region (Hanover) showed lower boldness than those from other regions, including
394 regions within the same genetic cluster. Altogether, these results suggest that both genetic clusters,
395 resulting from post-glacial colonization events and subsequent demographic processes, and
396 contemporary selection at the regional scale shaped the proactive behaviour of tadpoles. This is in
397 line with our previous studies showing a complex interplay between effects of historical and
398 contemporary processes on the genetic structure (Cortazar Chinarro et al., 2017; 2018, Meyer-
399 Lucht et al., 2019, Rödin-Mörch et al., 2019) and larval life history traits (Luquet et al., 2019) of
400 *R. arvalis* populations along the latitudinal gradient. These findings likewise align with the broader
401 framework of POLS theory, which proposes that fast-slow life history strategies correlate with
402 personality traits along environmental gradients (Reale et al. 2010). Our results on *R. arvalis*
403 tadpoles provide empirical support for this concept, particularly in the context of ectotherms facing
404 strong seasonal time constraints at early developmental stages, as previously observed in anti-
405 predator responses across lizard species (Samia et al. 2015). However, an interplay on proactive
406 behaviour of *R. arvalis* tadpoles is difficult to disentangle and seems also depend on the

407 behavioural trait considered and regional environmental conditions. Indeed, activity of tadpoles is
408 strongly influenced at the cluster scale with low variation within genetic cluster. In contrast,
409 tadpole boldness showed strong differences among regions within and between genetic clusters,
410 resulting in no difference in tadpole boldness between the genetic clusters. Tadpole exploration
411 was not influenced by latitude neither at genetic cluster nor at regional levels. This suggests that
412 boldness variation along the latitudinal gradient results from contemporary selection due to
413 environmental constraints for bolder genotypes in the northern populations, while activity
414 variation along the gradient results from the genetic divergence due to historical post-
415 recolonization. Findings of trait-specific behavioural responses in our latitudinal gradient study
416 further contribute to revisitations of POLS theory, demonstrating that different personality traits
417 can evolve independently in response to the influence of both historical events and ongoing
418 environmental pressures (Dingemanse et al., 2010; Wolf and Weissing, 2012, Royauté et al. 2018).

419

420 **Decoupling of proactive behaviours across ontogeny along the latitudinal gradient**

421 Interestingly, the proactive behaviour of froglets along the gradient is almost the reverse of the
422 tadpole behaviour. Froglets from the North cluster exhibited reduced boldness and exploration as
423 compared to the South, and froglets from the two northernmost regions were shyer and explored
424 less than froglets from other regions. There was no change in froglet activity along the gradient.
425 This result aligns well with the extended POLS theory, predicting that behaviours on the proactive-
426 reactive continuum can be decoupled across ontogeny (Montiglio et al., 2018). The observed
427 decoupling is likely due to fluctuating selective pressures that amphibians face as they transition
428 from aquatic to terrestrial environments following metamorphosis (Van Buskirk, 2002; Relyea and
429 Hoverman, 2003, Orizaola and Braña, 2005). Our results suggest that in high latitude populations,

430 tadpoles may benefit from increased boldness and activity in aquatic environments, where rapid
431 growth and resource acquisition are critical for survival as the tadpoles have to metamorphose
432 before the onset of winter (Laugen et al., 2003, Luquet et al., 2019, Orizaola et al., 2013). However,
433 once living in terrestrial environments, the cost of being overly bold or explorative in harsher
434 northern climates could outweigh the benefits. Specifically, in high-latitude populations time for
435 foraging after metamorphosis may be very short due to approaching winter which may select for
436 lower boldness and exploration activity. Indeed, the highest exploration level was found in the
437 Hanover population which experiences the longest season length along the gradient, while Luleå
438 experiences the shortest, suggesting that this trait may be related to the time available for resource
439 acquisition after metamorphosis. In juvenile *R. arvalis* terrestrial growth rates in laboratory are
440 lower in the northern than in the southern populations (Chondrelli, 2024), and studies on growth
441 and survival patterns in Scandinavian *Rana* populations suggest that survival is higher but
442 maturation occurs later in the northernmost populations (Hjernquist et al., 2012; Räsänen et al.,
443 2008; Söderman, 2006). These results suggest that time constraints for terrestrial growth and
444 development may be relaxed along the latitudinal gradient as compared to the more stringent
445 constraints faced by tadpoles, possibly allowing for decoupling of behaviours related to resource
446 acquisition (Sih et al., 2004). A recent study also suggests that the ontogenetic experience of
447 predator presence led to personality emergence in *R. arvalis* tadpoles and that this pattern was lost
448 over metamorphosis (Plaskonka et al. 2024); as predation pressure is known to decrease with
449 latitude (Laurila et al., 2008; Schemske et al., 2009) and could also influence the decoupling of
450 behaviours. Such ontogenetic decoupling of behaviours has been observed in other species, where
451 selective pressures shift as individuals transition between ecological niches (Wuerz and Kruger,
452 2015, Katsis et al. 2023, Class and Brommer, 2015, Bosco et al., 2017). Thus, our findings

453 underscore the importance of considering both life-history stage and the ecological context when
454 examining behavioural traits and personalities across geographical ranges.

455

456 **Relationship between proactive behaviours and larval growth along the latitudinal gradient**

457 Developmental growth is an important life history trait in larval amphibians, and several studies
458 have shown that this trait readily diverges along climatic gradients (e.g., Berven et al. 1983: Laurila
459 et al. 2008, Orizaola et al. 2010). In our previous study focusing on life-history traits, we observed
460 notable differences in larval growth among *R. arvalis* populations deriving from the two post-
461 glaciation colonization routes, with populations from the northern cluster showing a higher growth
462 rate (Luquet et al. 2019; see Figure 3). Behavioural variation of tadpoles observed in this study
463 (mainly higher activity in the North cluster and the northernmost regions, and higher boldness in
464 the northernmost region) appears to globally align with expectations that proactive behaviours
465 coevolve with faster larval growth. However, detailed relationships between proactive behaviours
466 and larval growth showed a strong influence of both genetic cluster and developmental stage.
467 Indeed, larval growth is positively associated with boldness in tadpoles from the South cluster but
468 not in tadpoles from the North one, while in froglets larval growth is positively associated with
469 boldness in the North cluster but not in the South one. At the regional scale, our findings further
470 disentangle the role of historical processes and local adaptation in shaping growth-behaviour
471 relationships. Positive associations between tadpole boldness and larval growth were observed in
472 Uppsala and Skåne, as well as between froglet activity and larval growth in Uppsala. However,
473 these associations were absent in other regions, suggesting that local environmental conditions
474 have more strength in modulating such associations, as previously observed in insects (Golab et
475 al. 2022).

476 As mentioned above, these different relationships across genetic clusters and among regions are
477 likely driven by both colonization history and climate-induced time constraints, which have
478 together shaped crucial shifts in metabolism and life-history traits (Laurila et al. 2008; Orizaola et
479 al. 2013, Caruso et al. 2020). Additionally, ecological factors such as predation pressure and
480 susceptibility to parasitic infections likely play crucial roles in shaping contrasting relationships
481 between life history and behaviour in different geographical areas. For instance, as predation
482 pressures on amphibians is typically reduced, and parasite diversity tends to decrease with
483 increasing latitude (Roslin et al. 2017, Rhode 1999), the contrasting relationships between growth
484 and proactivity across clusters may reflect the interplay of these ecological pressures. In the North
485 cluster, where both predation and parasitism are reduced, proactive behaviours may provide an
486 advantage later in development, explaining the positive association between boldness and larval
487 growth. Conversely, in the South cluster, where predation and parasite diversity are higher,
488 selection might favour proactivity earlier during tadpole stage, enabling faster growth and
489 providing an adaptive strategy that compensate higher exposure to predators and infection. This
490 divergence in selective pressures likely explains the inconsistencies observed between the genetic
491 clusters, with local adaptation influencing how behaviour and life-history traits co-vary across
492 ontogeny and different regions along the gradient.

493

494 **Conclusions**

495 *R. arvalis* populations along a broad latitudinal gradient in northern Europe exhibit clear
496 differences in proactivity levels, showing reversed behavioural patterns before and after
497 metamorphosis. Our findings suggest that natural selective pressures acting on behaviours along
498 the proactive-reactive continuum and life history traits operate differently across developmental

stages in this species. These results emphasize the importance of latitudinal gradient studies to elucidate evolutionary processes driving phenotypic variation in behaviour among natural populations. Future research should investigate the role of temperature, predator diversity, and resource availability in shaping these behaviours. Furthermore, linking behavioural variation across developmental stages to specific genomic polymorphisms could provide valuable insights into evolutionary processes, historical selection and the maintenance of behavioural diversity across regions.

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Author contributions

M.C-C.**;** writing original draft, writing-review and editing, A.C-L.; data curation, formal analyses, writing original draft writing-review 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. Consistency of individual behaviour. (A) Individual differences in measurements of time to emerge from the shelter taken during two independent trials performed to moor frog tadpoles (n = 30, dark green) and froglets (n= 33, light green) raised in a common garden experiment sampled across multiple regions of a 1700km latitudinal gradient in northern Europe. (B) Distribution of adjusted repeatability estimates of an individual time to emerge from the shelter in bootstrapping tests using a linear mixed model framework after controlling for effects introduced by region (see Stoffel et al. 2017). Blue dot indicates mean adjusted repeatability with confidence intervals for time to emerge in tadpoles (top) and froglets (bottom).

Figure 2. Behaviour of moor frogs across the latitudinal gradient. Boldness (A), activity (B) and exploration (C) measured in moor frog tadpoles (dark green) and froglets (light green) across a 1700 latitudinal gradient and raised in a common garden experiment (South genetic cluster: Hanover (Germany), red dot; Skåne (Sweden), yellow triangle; Uppsala, green square); North genetic cluster: Umeå (Sweden), blue cross; Luleå (Sweden), pink crossed square). Text indicates p-values of significance tests comparing difference in behaviour between genetic clusters (see Table 2; ns $p>0.05$, * $p<0.05$ ** $p<0.01$ *** $p<0.001$). Mean value and standard error of boldness (D), activity (E) and exploration (F) of tadpoles (dark green squares) and froglets (light green diamonds) for the five regions sampled. Average values for regions not sharing any letter are significantly different ($p < 0.05$) in post-hoc contrasts of independent models assessing differences in behaviour across regions for tadpoles (dark green letters) and froglets (light green letters; see Table 3-4).

755

756 **Figure 3. Association between behaviour and life history in moor frogs across the latitudinal**
757 **gradient.** Relationships between (A) boldness, (B) activity and (C) exploration and developmental
758 growth (mass/time at Gosner stage 42) in moor frog tadpoles (top row) and froglets (bottom row)
759 sampled across a 1700 latitudinal gradient and raised in a common garden experiment (South
760 genetic cluster: Hanover (Germany), red dot; Skåne (Sweden), yellow triangle; Uppsala, green
761 square); North genetic cluster: Umeå (Sweden), blue cross; Luleå (Sweden), pink crossed square).
762 Trend lines and confidence interval are displayed for the relationship between each behaviour and
763 developmental growth for values at the North (blue) and South (yellow) genetic clusters. Text
764 indicates estimated marginal means and standard error for each trend in post-hoc contrasts of
765 statistical models ($p < 0.005$ in bold; see Tables 5-6).

Table 1. Consistency of individual behaviour. Results for repeatability analyses of measurements of time to emerge from a shelter taken twice to moor frogs during tadpole stage (n = 30), and froglet stage (n= 33) raised in a common garden experiment following collection from multiple regions across a 1700km latitudinal gradient in northern Europe. Using a linear mixed model framework, we estimated adjusted repeatability of boldness for individual and population after controlling for effects introduced by region in the model ($_{adj}R$**;** see Stoffel et al. 2017), as well as the quantification of uncertainty for the variance explained by region of origin (R^2 coefficient, see Stoffel et al. 2017).

Developmental stage	**Factor**	**$_{adj}R$ / R^2**	***SE***	**95% CI**	**P**
Tadpole	Individual	0.00	0.06	[0, 0.226]	1.00
	Population	0.00	0.06	[0, 0.131]	1.00
	Fixed (region)	0.23	0.16	[0.088, 0.683]	-
Froglet	Individual	0.36	0.19	[0, 0.577]	**0.023***
	Population	0.00	0.18	[0, 0.535]	1.00
	Fixed (region)	0.24	0.18	[0.079, 0.769]	-

Table 2. Behavior across genetic clusters and developmental stages. Results for statistical analyses evaluating behavioral traits across tadpole and froglet stages in moor frogs originated from five different regions across a 1700 latitudinal gradient and raised in a common garden experiment (South genetic cluster: Hanover (Germany), Skåne (Sweden), Uppsala), North genetic cluster: Umeå (Sweden), Luleå (Sweden)). Boldness data was analyzed using a mixed effect survival model, while activity and exploration data was analyzed using linear mixed models. Full models included genetic cluster, developmental stage and interactions as fixed effects, while population of origin, region and individual identity as random intercept effects. Individual mass at the time of recording was added as a covariate.

	Boldness: Time to leave the shelter				**Activity:** mean speed				**Exploration:** mean distance to shelter			
Predictors	*Δ log-lik*	χ^2	*p*	*df*	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
cluster	16.0 ***	32.04	**<0.001**	1	0.04 (0.04)	1.33	0.32	$1_{3.23}$	785.1 (785.1)	6.02	0.064	$1_{4.42}$
stage	-31.5 ***	137.03	**<0.001**	1	1.96 *** (1.96)	61.07	**<0.001**	$1_{466.5}$	716.5 * (716.5)	5.50	**0.019**	$1_{412.8}$
mass at recording	2.6 *	5.22	**0.022**	1	0.14* (0.13)	4.23	**0.040**	$1_{504.3}$	157.0 (157.0)	1.21	0.27	$1_{409.7}$
cluster × stage	14.5 ***	28.98	**<0.001**	1	1.10*** (1.10)	34.26	**<0.001**	$1_{310.6}$	1164.6** (1164.6)	8.94	**0.003**	$1_{339.9}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*	*Δ log-lik*	χ^2	*p*	*df*	*Δ log-lik*	χ^2	*p*	*df*
id	0.0	0.00	0.97	1	-1.4	2.77	0.096	1	0.0	1.81	0.18	1
population	0.8	1.66	0.19	1	3.0 *	6.07	**0.013**	1	0.0	0.0	1	1
region	0.0	0.05	0.81	1	0.2	0.55	0.45	1	0.0	0.13	0.71	1
σ^2					0.03				130.28			
τ_{00}	0.00_{id}				0.01_{id}				14.62_{id}			
	$0.01_{population}$				$0.00_{population}$				$0.00_{population}$			

	0.00_{region}	0.00_{region}	2.09_{region}
Observations	655	512	512
Marginal R^2	0.71	0.365	0.058

** p<0.05 ** p<0.01 *** p<0.001*

Table 3. Behavior across regions and developmental stages. Results for statistical analyses evaluating behavioral traits across tadpole and froglet stages in moor frogs originated from five different regions across a 1700 latitudinal gradient and raised in a common garden experiment (Hanover (Germany), Skåne (Sweden), Uppsala), Umeå (Sweden), Luleå (Sweden)). Boldness data was analyzed using a mixed effect survival model, while activity and exploration data was analyzed using linear mixed models. Full models included developmental stage, region and its interaction as fixed effects, while population of origin, and individual identity as random intercept effects. Individual mass at the time of recording was added as a covariate.

	Boldness: Time to leave the shelter				**Activity:** mean speed				**Exploration:** mean distance to shelter			
Predictors	*Δ log-lik*	χ^2	*p*	*df*	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
region	29.2 *	58.58	**<0.001**	1	0.23 (0.06)	1.80	0.20	$4_{11.1}$	2606.1 ** (651.5)	5.23	**0.007**	$4_{16.5}$
stage	78.9 ***	156.29	**<0.001**	1	1.53 *** (1.53)	48.05	**<0.001**	$1_{453.4}$	204.2 (204.2)	1.65	0.20	$1_{455.2}$
mass at recording	2.3 *	4.73	**0.030**	1	0.12 * (0.12)	3.91	**0.048**	$1_{500.4}$	163.7 (163.7)	1.32	0.25	$1_{450.1}$
region × stage	24.3 ***	48.66	**<0.001**	1	1.24 *** (0.31)	9.71	**<0.001**	$4_{288.0}$	3511.7 *** (877.7)	7.09	**<0.001**	$4_{309.7}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*	*Δ log-lik*	χ^2	*p*	*df*	*Δ log-lik*	χ^2	*p*	*df*
id	0.0	0.00	0.95	1	1.6	3.25	0.071	1	-0.8	2.40	0.12	1
population	0.0	0.08	0.77	1	1.4	2.74	0.098	1	0.0	0.00	1	1
σ^2					0.03				123.83			
τ_{00}	0.00_{id}				0.01_{id}				16.85_{id}			
	$0.00_{population}$				$0.00_{population}$				$0.00_{population}$			
Observations	655				512				512			
Marginal R^2	0.725				0.389				0.104			

** p<0.05 ** p<0.01 *** p<0.001*

Table 4. Influence of behavior on life history (growth rate) across genetic clusters and developmental stages. Results for statistical analyses evaluating growth rate in moor frog tadpoles and froglets originating from five different regions across a 1700 latitudinal gradient and raised in a common garden experiment (South genetic cluster: Hanover (Germany), Skåne (Sweden), Uppsala), North genetic cluster: Umeå (Sweden), Luleå (Sweden)). Growth rate linear mixed models included the behavior of interest, genetic cluster, developmental stage and their interaction as predictors, and population of origin and and region as random intercept effects.

Growth rate including boldness (time to leave the shelter) as predictor

Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Boldness	0.18 (0.18)	0.57	0.45	$1_{620.5}$
cluster	67.65 *** (67.65)	205.0	**<0.001**	$1_{41.8}$
stage	0.44 (0.44)	1.35	0.25	$1_{618.4}$
Boldness × cluster	1.79 ** (1.79)	5.44	**0.020**	$1_{620.4}$
Boldness × stage	0.17 (0.17)	0.52	0.48	$1_{620.3}$
cluster ×stage	0.74 (0.74)	2.24	0.13	$1_{618.4}$
Boldness × cluster× stage	2.63 ** (2.63)	7.96	**0.004**	$1_{620.3}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	38.0 ***	16.1	**<0.001**	1
region	0.0	0	1	1

σ^2	0.33
ICC	0.06
Observations	631
Marginal R^2	0.647

Growth rate including activity (mean speed) as predictor

Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Activity	0.49 (0.49)	1.47	0.22	$1_{475.2}$
cluster	21.09 *** (21.09)	62.36	**<0.001**	$1_{166.5}$
stage	0.88 (0.88)	2.62	0.11	$1_{476.2}$
Activity × cluster	0.00 (0.00)	0.00	0.98	$1_{475.2}$
Activity × stage	0.42 (0.42)	1.25	0.27	$1_{474.0}$
cluster ×stage	0.04 (0.04)	0.11	0.74	$1_{476.1}$
Activity × cluster× stage	0.07 (0.07)	0.02	0.89	$1_{474.0}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	6.1 ***	11.5	**<0.001**	1
region	0.0	0	1	1

σ^2	0.34
ICC	0.06
Observations	489
Marginal R^2	0.631

Growth rate including exploration (mean distance to shelter) as predictor

Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Exploration	0.02 (0.02)	0.07	0.80	$1_{474.2}$
cluster	77.23 *** (77.23)	230.52	**<0.001**	$1_{16.5}$
stage	1.68* (1.68)	5.02	**0.025**	$1_{479.1}$
Exploration × cluster	0.01 (0.01)	0.04	0.84	$1_{474.2}$
Exploration × stage	2.12 * (2.12)	6.34	**0.01**	$1_{475.8}$
cluster ×stage	0.37 (0.37)	1.10	0.29	$1_{479.1}$
Exploration × cluster× stage	0.95 (0.95)	2.84	0.092	$1_{475.8}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	7.7 ***	12.5	**<0.001**	1

region		0.0	0	1	1
σ^2	0.34				
ICC	0.07				
Observations	489				
Marginal R^2	0.634				

** p<0.05 ** p<0.01 *** p<0.001*

Table 5. Influence of behavior on life history (growth rate) across regions and developmental stages. Results for statistical analyses evaluating growth rate in moor frog tadpoles and froglets originating from five different regions across a 1700 latitudinal gradient and raised in a common garden experiment (from South to North: Hanover (Germany), and Skåne, Uppsala, Umeå, Luleå (Sweden)). Growth rate models included the behavior of interest, region, developmental stage and their interaction as predictors, and population of origin as a random intercept effect.

Growth rate including boldness (time to leave the shelter) as predictor

Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Boldness	0.08 (0.08)	0.24	0.62	$1_{620.5}$
region	60.94 *** (15.23)	46.71	**<0.001**	$1_{41.8}$
stage	0.28 (0.28)	0.85	0.36	$1_{618.4}$
Boldness × region	5.30 ** (1.32)	4.06	**0.030**	$1_{620.4}$
Boldness × stage	0.13 (0.13)	0.41	0.52	$1_{620.3}$
region ×stage	2.90 (0.73)	2.21	0.065	$1_{618.4}$
Boldness × region× stage	7.07 *** (1.77)	5.42	**<0.001**	$1_{620.3}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	5.88 ***	11.8	**<0.001**	1
σ^2 0.33				
ICC 0.07				

Observations	631
Marginal R^2	0.651

Growth rate including activity (mean speed) as predictor

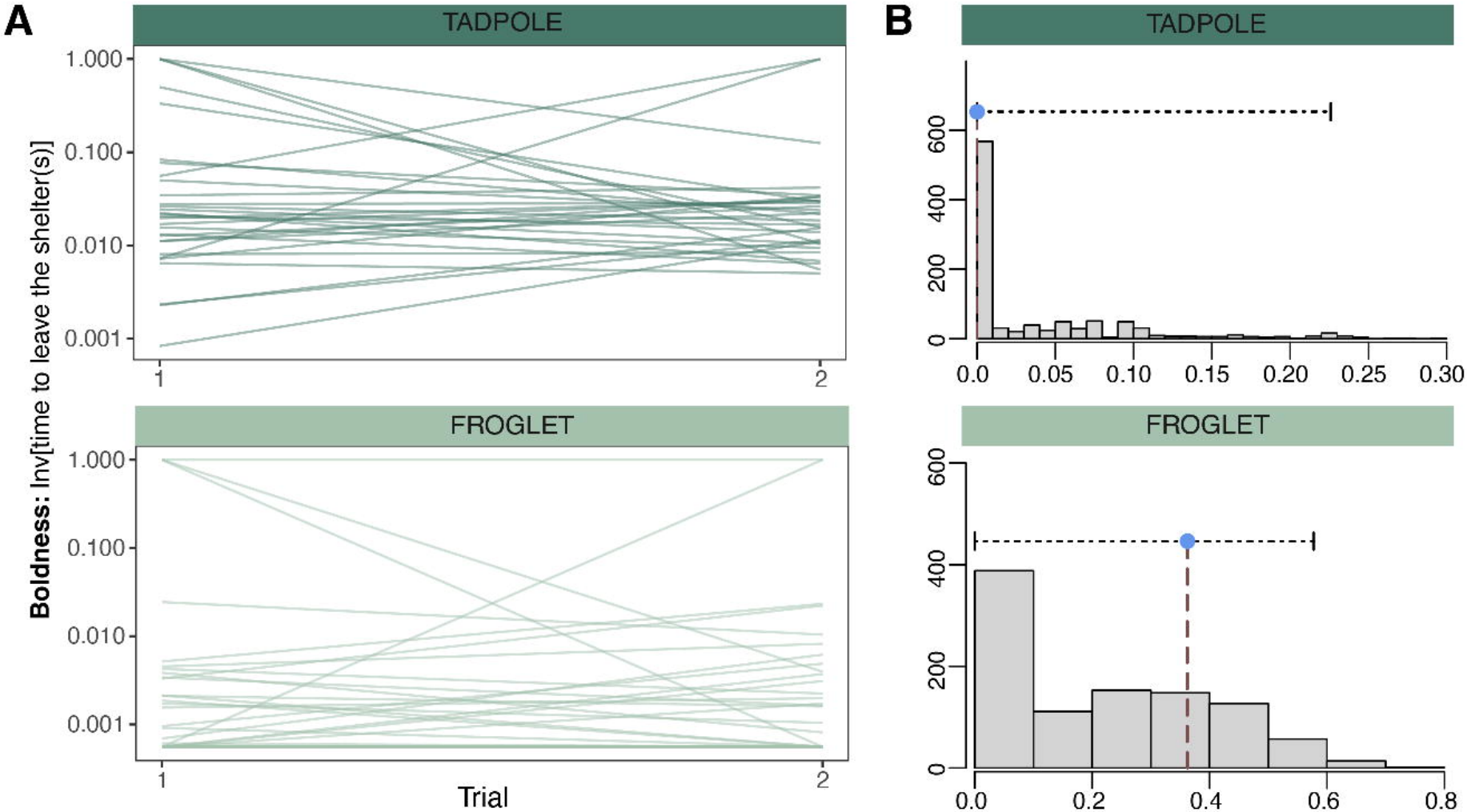
Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Activity	0.13 (0.13)	0.38	0.54	$1_{463.0}$
region	25.33 *** (6.33)	19.10	**<0.001**	$4_{53.0}$
stage	0.64 (0.64)	1.93	0.16	$1_{463.1}$
Activity × region	5.07 ** (1.29)	3.83	**0.005**	$4_{462.2}$
Activity × stage	0.13 (0.42)	0.39	0.53	$1_{462.9}$
region ×stage	3.94 * (0.99)	2.97	**0.019**	$4_{462.9}$
Activity × region× stage	5.04 ** (1.26)	3.80	**0.005**	$4_{463.0}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	3.6 **	7.2	**0.071**	1
σ^2	0.33			
ICC	0.07			
Observations	489			

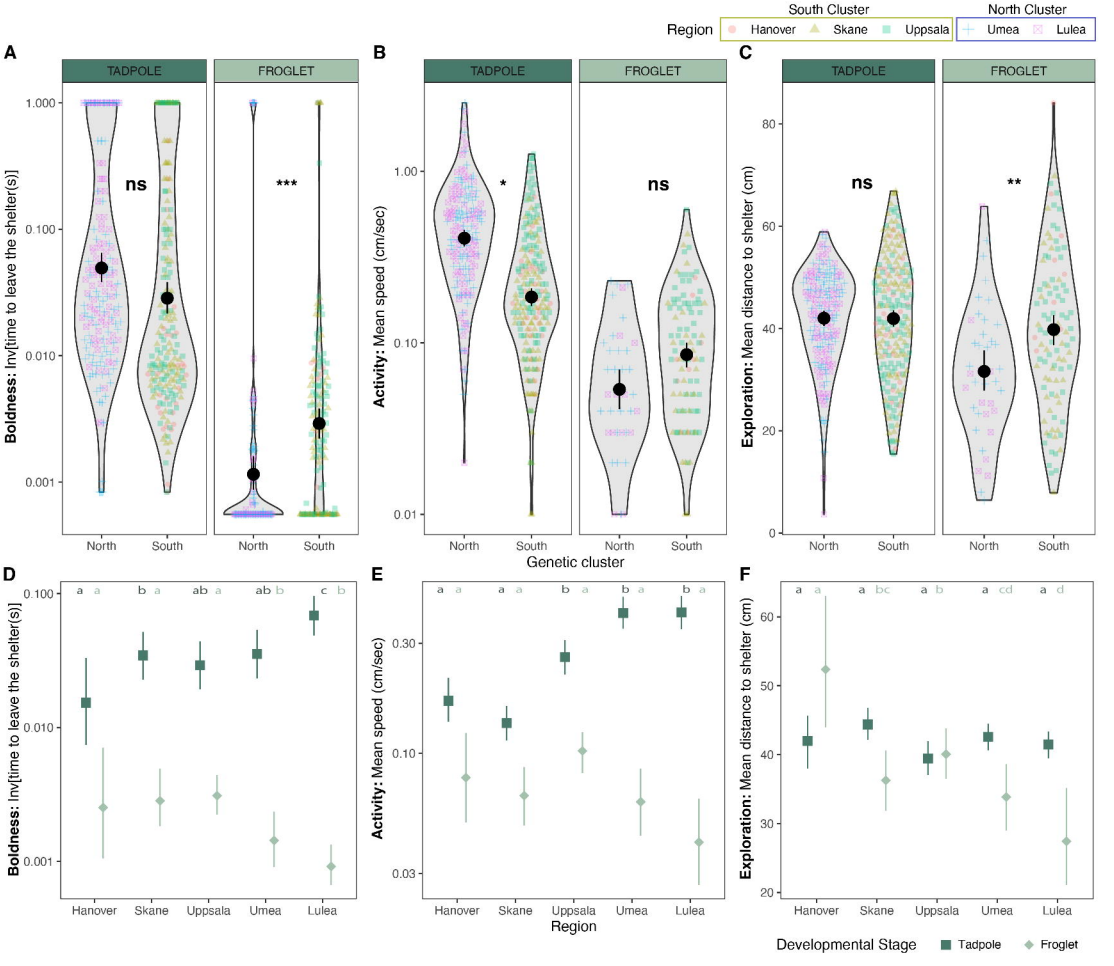
Marginal R^2	0.639

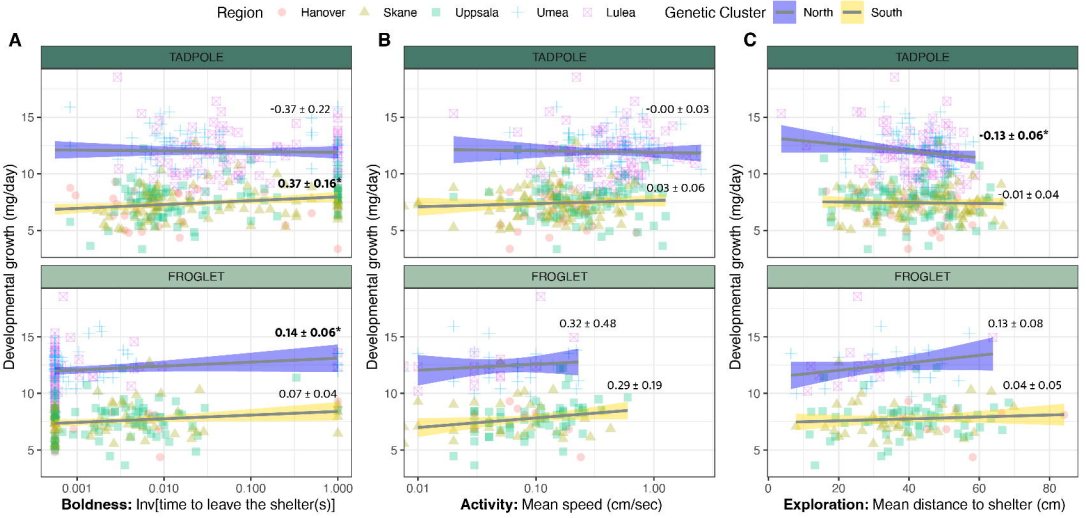
Growth rate including exploration (mean distance to shelter) as predictor

Predictors	*SumSq (mean sq)*	*F-value*	*p*	*df (Num$_{dem}$)*
Exploration	0.00 (0.0)	0.01	0.92	$1_{461.8}$
region	69.78 *** (17.45)	51.71	**<0.001**	$4_{11.5}$
stage	2.09 * (2.09)	6.21	**0.013**	$1_{463.6}$
Exploration × region	0.68 (0.17)	0.50	0.74	$4_{462.0}$
Exploration × stage	1.87 * (1.87	5.54	**0.019**	$1_{463.5}$
region ×stage	1.39 (0.35)	1.03	0.38	$4_{463.2}$
Exploration × region× stage	1.50 (0.37)	1.11	0.35	$4_{463.3}$
Random Effects	*Δ log-lik*	χ^2	*p*	*df*
population	3.9 ***	7.7	**0.005**	1
region	0.0	0	1	1
σ^2	0.34			
ICC	0.07			
Observations	489			
Marginal R^2	0.634			

** p<0.05 ** p<0.01 *** p<0.001*


A
TADPOLE
FROGLET
Boldness: Inv[time to leave the shelter(s)]
1.000
0.100
0.010
0.001
1
2
Trial
B
TADPOLE
FROGLET
0
200
400
600
0.0
0.05
0.10
0.15
0.20
0.25
0.30
0.2
0.4
0.6
0.8


Region
South Cluster
Hanover
Skane
Uppsala
North Cluster
Umea
Lulea
A
B
C
TADPOLE
FROGLET
Boldness: Inv[time to leave the shelter(s)]
Activity: Mean speed (cm/sec)
Exploration: Mean distance to shelter (cm)
ns
***
*
**
North
South
Genetic cluster
D
E
F
Hanover
Skane
Uppsala
Umea
Lulea
Region
Developmental Stage
Tadpole
Froglet


Region
Hanover
Skane
Uppsala
Umea
Lulea
Genetic Cluster
North
South
A
B
C
TADPOLE
FROGLET
Developmental growth (mg/day)
-0.37 ± 0.22
0.37 ± 0.16*
0.14 ± 0.06*
0.07 ± 0.04
-0.00 ± 0.03
0.03 ± 0.06
0.32 ± 0.48
0.29 ± 0.19
-0.13 ± 0.06*
-0.01 ± 0.04
0.13 ± 0.08
0.04 ± 0.05
Boldness: Inv[time to leave the shelter(s)]
Activity: Mean speed (cm/sec)
Exploration: Mean distance to shelter (cm)