Boldness and exploration are linked to shell morph but not environmental context in the snail Cepaea nemoralis — Source link ↗

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1 **Boldness and exploration vary between shell morphs but not environmental**

2 **contexts in the snail *Cepaea nemoralis***

3 **Running title:** Personality and shell morph in snails

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9

10 **Acknowledgments**

14 **Conflicts of interest**

15 The authors have no conflict of interest to declare.

16 **Data accessibility**

17 Data and code to reproduce all analyses are available on GitHub

18 (https://github.com/mdahirel/cepaea-personality-2017) and archived in Zenodo (DOI:

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1

22   **Abstract**

23   Understanding the maintenance of among-individual behavioral variation in populations, and

24   predicting its consequences, are key challenges in behavioral ecology. Studying the association

25   between repeatable behaviors and other traits under selection may shed light on the underlying

26   selective pressures. We used the model snail *Cepaea nemoralis* to examine whether individual

27   behavior is associated with shell morph, a key trait that has been extensively studied in the context

28   of thermal tolerance and predator avoidance, and which is known to be under strict genetic control

29   in this species. We quantified proxies of boldness and exploration in snails of three morphs coming

30   from two habitats with different thermal contexts. We show that both behaviors were repeatable at

31   the among-individual level (within-state $R_{boldness}$ = 0.22 [95% credible interval: 0.15, 0.29]; $R_{exploration}$ =

32   0.20 [0.15, 0.25]). Behavior was associated with shell morph, with the darker morph (five-banded)

33   being consistently shyer and slower to explore. There was no evidence that thermal environment of

34   origin influenced behavior. Snails became faster when test temperature increased; we found no

35   evidence morphs differed in their thermal response. Boldness and exploration were correlated

36   among individuals, forming a syndrome (r = 0.28 [0.10, 0.46]). We discuss what these results may tell

37   us about the type of selection exerted by predators. We also detail how our results hint to a genetic

38   link between shell morph and behavior, and the evolutionary implications of such a link. Finally, we

39   discuss how our findings combined with decades of evolutionary research make *C. nemoralis* a very

40   valuable model to study the evolution of behavior in response to environmental changes.

41   **Keywords** animal personality; behavioral syndromes; Gastropoda; multivariate multilevel model;

42   shell color; temperature

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43   **Introduction**

44   A key question in behavioral ecology, and more broadly in evolutionary ecology, is how to explain the

45   persistence of variation in phenotypic traits. Although behavior is often seen as highly labile and

46   dynamically adjustable to experienced conditions, individuals of many animal species exhibit

47   "personalities", i.e. behave consistently across time and contexts, and differ consistently from each

48   other (Kralj-Fišer & Schuett, 2014; Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Sih, Bell,

49   Johnson, & Ziemba, 2004). This among-individual variation persists even when better adjustments of

50   behaviors to environmental conditions would be adaptive, and the ability to tune behavior to

51   conditions may itself vary among individuals (variation in "behavioral reaction norms"; Dingemanse,

52   Kazem, Réale, & Wright, 2010). Moreover, behaviors are often correlated with each other and with

53   other traits, forming multivariate syndromes (Réale et al., 2007; Royauté, Berdal, Garrison, &

54   Dochtermann, 2018; Santostefano, Wilson, Niemelä, & Dingemanse, 2017), further constraining the

55   range of behavioral phenotypes that are on display in populations (Dochtermann & Dingemanse,

56   2013).

57   State-dependent behavior is often invoked as one of the key mechanisms/frameworks potentially

58   explaining both adaptive correlations/feedbacks between behaviors and other traits, and the

59   maintenance of among-individual variation (Sih et al., 2015; Wolf & McNamara, 2012; Wolf &

60   Weissing, 2010). Individuals can differ in morphology, size, past experienced environment, or any

61   other so-called "state variables", typically less labile than behavior or even fixed at the individual

62   level. If the costs and benefits of behaviors vary depending on these state variables, then we should

63   expect individuals differing in state to adaptively differ in behaviors as well (Wolf & Weissing, 2010).

64   The pace-of-life hypothesis, which ties several axes of behavioral variation to underlying differences

65   in life history and metabolism along a fast-slow axis (Réale et al., 2010; Wolf & McNamara, 2012;

66   Wright, Bolstad, Araya-Ajoy, & Dingemanse, 2019), can be seen under this lens. Other examples

67   include cases of phenotypic compensation, where predation risk can either be mitigated by

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68   behavioral changes or morphological defenses, leading to a positive association between risk-taking

69   behavior and defenses (e.g. Ahlgren, Chapman, Nilsson, & Brönmark, 2015; but see De Winter,

70   Ramalho Martins, Trovo, & Chapman, 2016 for a contradictory example). In some cases, quantitative

71   genetics and/or experimental evolution approaches may provide evidence of the evolution of state-

72   behavior associations (e.g. Kern, Robinson, Gass, Godwin, & Langerhans, 2016). In other cases in

73   which this may be difficult, we believe that studying the association between personality and state

74   traits can still provide valuable insights, especially if (i) the state trait is known to be fully genetically

75   determined with little to no plasticity, (ii) we are able to study behavioral variation across a range of

76   environments known to select on the state variable.

77   The grove snail *Cepaea nemoralis* (Linnaeus 1758) (family Helicidae) is a simultaneous

78   hermaphrodite, medium-sized land gastropod common in western Europe (adult shell diameter 18-

79   25mm; Welter-Schultes, 2012). It has a long history as a model in evolutionary biology, due to its

80   conspicuous shell polymorphism (reviewed by Jones, Leith, & Rawlings, 1977; Ożgo, 2009)(Fig. 1A-B).

81   Genetic variation in shell background color (from pale yellow to brown, but usually divided in yellow,

82   pink, and brown; Davison, Jackson, Murphy, & Reader, 2019) and in the number or width of dark

83   bands has been well described (Jones et al., 1977). Shell polymorphism is governed by a limited

84   number of loci with a limited number of alleles (Richards et al., 2013), and by all evidence shows no

85   phenotypic plasticity. Modern genomics studies now aim to pinpoint the actual

86   molecular/physiological underpinnings of shell color (Kerkvliet, Boer, Schilthuizen, & Kraaijeveld,

87   2017; Richards et al., 2013).

88   In *C. nemoralis*, lighter-colored shells absorb less heat and allow snails to maintain a lower body

89   temperature (Heath, 1975) and higher water content (Chang, 1991). Many studies have shown that

90   lighter (vs. darker) snails have a selective advantage in hotter/sunnier (vs. colder/shaded)

91   environments, whether one looks at continental-scale latitudinal clines (Jones et al., 1977; Silvertown

92   et al., 2011), local-scale habitat comparisons (Kerstes, Breeschoten, Kalkman, & Schilthuizen, 2019;

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93   Ozgo & Kinnison, 2008; Schilthuizen, 2013), or historical comparisons in the context of climate

94   change (Ożgo, Liew, Webster, & Schilthuizen, 2017; Ożgo & Schilthuizen, 2012). Local variations in

95   morph frequencies have also been linked to predation pressure, generally in the context of visual

96   selection (frequency-dependent selection and/or crypsis; Jones et al., 1977; Surmacki, Ożarowska-

97   Nowicka, & Rosin, 2013, and references therein; but see Cook, 2008), but morph differences in shell

98   resistance to crushing have also been described (Rosin, Kobak, Lesicki, & Tryjanowski, 2013).

99   Although this has been much less studied, shell morphs also vary in their behavior (Chang, 1991;

100   Jones, 1982; Ożgo & Kubea, 2005; Rosin et al., 2018). Part of this variation is likely the direct

101   consequence of differences in shell thermal properties and thus effective body temperature, as

102   demonstrated by experiments that created "artificial" morphs by painting shells (Tilling, 1983).

103   However, they probably also reflect, at least partly, intrinsic physiological differences: preferred

104   temperatures can be altered using opioid agonists or antagonists, but banded snails are less

105   responsive to this pharmaceutical manipulation (Kavaliers, 1992). Existing studies, however, have

106   several major shortfalls for our understanding of the association between *Cepaea* morphology and

107   behavioral syndromes. In particular, individuals are generally assayed once, which means separating

108   within- from among-individual variation is impossible (Dingemanse & Wright, 2020; Niemelä &

109   Dingemanse, 2018). This also means the level of total among-individual variation, and how it

110   compares with among-morph variation, has remained to our knowledge unstudied. Additionally, all

111   snails are often sampled from the same habitat, or habitat information is not used in behavioral

112   analyses, meaning there is often no way to determine how behavior responds to selection pressures

113   on shell color.

114   In this context, we investigated the existence and magnitude of personality variation and behavioral

115   syndromes in *C. nemoralis*, how behavior is linked to shell variation, and how it is influenced by the

116   environment of origin (sun-exposed or shaded) and currently experienced conditions. More

117   specifically, we make the following hypotheses:

5

118   (i) Exploration and boldness (risk-taking behavior) are both repeatable in this species, and positively

119   correlated in a behavioral syndrome (Réale et al., 2010).

120   (ii) As boldness may increase predation risk (e.g. Hulthén et al., 2017), we may expect phenotypic

121   compensation through shell characteristics to be present in bolder individuals. This would lead to

122   correlations between morph and behavior, the direction of which providing insights into the

123   dominant selection pressures.

124   (iii) As snails are ectotherms, exploration should increase with temperature due to increased

125   metabolism (over the range of temperatures suitable to movement; Abram, Boivin, Moiroux, &

126   Brodeur, 2017; Cloyed, Dell, Hayes, Kordas, & O'Gorman, 2019). We expect this temperature-

127   exploration reaction norm should vary both in its slope and average value according to shell morph

128   and habitat of origin. Populations having evolved in sun-exposed habitats, and lighter (unbanded)

129   snails should be better adapted to maintain activity in the face of high temperatures (e.g. Cloyed et

130   al., 2019), at the possible costs of lower activity at lower temperatures (Tilling, 1983).

131   **Methods**

132   *Sampled sites and snail maintenance under laboratory conditions*

133   Snails were sampled in fall 2016 in and close to the village of Arçais, France (Fig. 1C), roughly in the

134   middle of the recorded latitudinal range of *Cepaea nemoralis* (GBIF Secretariat, 2020). We studied

135   two sites located about 2 km apart and differing in terms of vegetation cover. One was a garden with

136   few isolated trees, and thus under relatively direct sun exposure all year long (hereafter the "open

137   habitat"; approximate location: 46° 17' 50"N, 0° 41' 30" W, Fig. 1D). The other was a 200 by 150 m

138   deciduous forested lot, and thus fully shaded a large part of the year, especially the hottest spring

139   and summer months ("shaded habitat"; approximately 46° 18' 01" N, 0° 42' 56" W, Fig. 1E). Only

140   adult snails were selected (recognizable by a reflected "lip" on their shell opening), as a way to partly

141   control for age. We only sampled snails with the three most abundant shell banding patterns: shells

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142 with no bands, three bands on the lower side of the shell, and five bands (Fig. 1B). Following previous

143 authors (e.g. Kavaliers, 1992), we here focused for simplicity solely on band presence, and thus

144 sampled only snails with yellow background shells, which are the most common in the study region

145 (Silvertown et al., 2011; personal observations) and on which the contrast between shell background

146 and dark bands is the strongest. We acknowledge that this may prevent us from fully generalizing, for

147 now, to natural populations, as the effect of darker background color is not always the same as the

148 effect of increased shell banding (e.g. Kerstes et al., 2019). Snails were hand-collected during the day,

149 their period of inactivity, both by simplicity and to avoid skewing our sample towards more active

150 individuals. If there were nonetheless a bias towards catching more conspicuous/ less likely to hide

151 snails, we believe it would have artificially reduced, rather than increased, our effect sizes: we would

152 have sampled the most active morph and the most active individuals from the least active morph,

153 reducing mean morph differences.

154 Sampling for the present study was targeted and adjusted in the field to obtain roughly equal

155 numbers of each banding pattern from both landscapes; it therefore did not allow us to make

156 inferences on their relative abundances. The same sites were however sampled again in 2018 for a

157 separate experiment, this time with random sampling relative to banding pattern. As in previous

158 studies (e.g. Schilthuizen, 2013) and reflecting potential thermal selection, the darker five-banded

159 snails were more frequent in the shaded habitat than in the open habitat (22.3 vs. 13.5 %; see

160 Supplementary Material S1).

161 We transferred snails to the lab and kept them under dormancy conditions (6 ± 1°C, no light, food or

162 water sources) until March 2017, about 3 weeks before the start of the experiment. We then divided

163 them into groups of 15 individuals from the same landscape, five (randomly chosen) of each shell

164 phenotype. Comparing group size to natural densities is difficult, due to the way natural densities are

165 often reported in the literature (averages over entire habitats, including empty areas). However,

166 groups of 10-20 individuals are commonly seen in the wild (personal observations) and are also often

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167   used in experiments (Oosterhoff, 1977; Rosin et al., 2018; Wolda, 1967). Groups were kept under

168   controlled conditions (20 ± 1°C, L:D 16:8) in 8.5 × 15 × 12 cm polyethylene boxes lined with 1-2 cm of

169   soil kept humid at the bottom. Snails had *ad libitum* access to prepared snail food (cereal flour

170   supplemented with calcium, Hélinove, Saint Paul en Pareds, France) in a Petri dish. We gave each

171   snail a unique ID written on the side of their shell with a paint marker (uni Posca, Mitsubishi Pencil

172   Co., Ltd, Tokyo, Japan; Henry & Jarne, 2007). A total of 360 snails (60 for each habitat × shell

173   phenotype combination) were used in the experiments described below. By necessity, the observer

174   (see below) was not blind to individual habitat of origin/phenotype; note that the analyst (MD) did

175   not contribute to the actual observations.

176   *Behavioral tests: boldness*

177   We studied boldness using simulated predator attacks as in Dahirel et al. (2017). All tests were done

178   by the same operator (VG) to avoid effects of inter-experimenter variability. Snails were assayed

179   individually during the last four hours of the photophase, i.e. the early part of the daily activity

180   period. Like other helicids, *Cepaea nemoralis* is nocturnal but tends to start activity sometime before

181   dark (Cameron, 1970). To stimulate activity, we first placed them in a Petri dish with water for 5

182   minutes, before putting them on individual clean glass plates. After snails had moved at least one

183   shell length (≈20 mm) from their starting position, the operator used a pipette tip to pinch them for 5

184   seconds on the right side of the foot. Preliminary tests confirmed that this was the shortest time

185   needed to ensure all snails retracted fully in their shell. We then recorded the time snails took to exit

186   the shell and resume activity after the attack (from retraction to the full extension of all tentacles out

187   of the shell), as our measure of boldness (snails with shorter latencies being considered bolder). We

188   stopped observations after 20 min if snails did not exit the shell. Snails from the same test box were

189   tested on the same day, and placed back in their box after testing. To estimate the repeatability of

190   boldness, snails were tested a second time after seven days, using the same protocol. The initial

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191   order in which groups were tested within a sequence was random; this order was conserved for all

192   subsequent tests.

193   *Behavioral tests: exploration/speed*

194   We studied snail movement at four temperatures within the activity range of *C. nemoralis* (Cameron,

195   1970): 15, 18, 22, and 25 °C. All tests were again performed by the same operator (VG), and again

196   during the last four hours of the photophase each day. Movement tests started 7 days after the last

197   boldness test for a given individual, successive movement tests were separated by 24h. Half of the

198   boxes, equally distributed between landscapes of origin, were tested in increasing temperature order

199   (from 15 to 25 °C), the other half in decreasing order (25°C to 15°). Twenty-four hours before a given

200   test, we placed snails and their rearing box at the testing temperature for habituation, using

201   temperature-controlled cabinets (ET 619-4, Lovibond, Dortmund, Germany). For testing, each snail

202   was placed individually at the center of a clean 25 × 25 cm polyethylene box (height: 9 cm) and left

203   free to move. Snails were deemed active once they had moved more than 2 cm away from their

204   starting point. We used the time snails took to move more than 10 cm from their starting point,

205   minus the time taken to start activity, as our exploration metric (with lower values for snails that

206   moved away faster). We stopped observations after 20 min post-activity initiation. This metric was

207   chosen for its ease of implementation; we acknowledge that it conflates exploration of the

208   environment with movement speed (as both slow-moving individuals and thorough explorers would

209   have higher first-passage times).

210   *Ethical note and compatibility with reporting guidelines*

211   This study complies with all relevant national and international laws, and the ASAB/ABS Guidelines

212   for the use of animals (2020) were adhered to as closely as possible. Potentially stressful

213   experimental treatments (boldness experiment) were limited to the shortest possible time to elicit

214   the behaviors of interest. No ethical board recommendation or administrative authorization was

215   needed to work on or sample *Cepaea nemoralis*. The marking method used is non-invasive and has

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216   minimal to no documented effects on life-history traits (Henry & Jarne, 2007). We do not believe

217   there is any potential for bias due to social background, self-selection, experience or other factors

218   indicated in the STRANGE framework (Webster & Rutz, 2020). To the best of our knowledge, the

219   studied individuals are representative of the local populations studied, except for the two constraints

220   explicitly imposed on collection by our experimental design (only adults, equal numbers of a few

221   morphs of interest). All individuals were subjected to the same experimental conditions once

222   collected.

223   *Statistical analyses*

224   We analyzed snail behavioral data in a Bayesian multilevel/mixed model framework, using the Stan

225   language (Carpenter et al., 2017), with R (version 4.0; R Core Team, 2020) and the *brms* R package

226   (Bürkner, 2017) as frontends. Scripting, analysis, and plotting relied on the *tidybayes*, *bayesplot*, and

227   *patchwork* packages, as well as the *tidyverse* family of packages (Gabry, Simpson, Vehtari,

228   Betancourt, & Gelman, 2019; Kay, 2019; Pedersen, 2019; Wickham et al., 2019).

229   We used a bivariate generalized linear multilevel model to estimate the effect of shell phenotype,

230   habitat and temperature on behavior, quantify behavioral (co)variances and partition them across

231   hierarchical levels (among-box, among-individual and within-individual variation) (Dingemanse &

232   Dochtermann, 2013; Houslay & Wilson, 2017). We did not estimate within-individual trait

233   correlations, as exploration and boldness were tested independently at the within-individual level

234   (that is, boldness measure 1 had no stronger "link" to exploration measure 1 than boldness measure

235   2; scenario 4 of table 2 in Dingemanse & Dochtermann, 2013). We present a full write-up of the

236   model as Supplementary Material S2; a general description follows below.

237   Boldness and exploration were analyzed assuming a lognormal distribution to account for the

238   skewed distribution of time to event data. We accounted for the fact that monitoring was stopped

239   before some individuals could express the behavior of interest by including a censored data indicator

240   in the model. Fixed effects for both behaviors included shell banding (three-level categorical

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241   variable), landscape of origin (binary variable), and their interaction, as well as test order (1 or 2 for

242   boldness, 1 to 4 for exploration). The model for exploration additionally included a test temperature

243   effect as well as its interactions with shell banding and landscape. Categorical variables (shell

244   banding, landscape of origin) were converted to centered dummy variables, and numeric variables

245   (test order, temperature) were centered, following Schielzeth (2010)(temperature was additionally

246   scaled to unit 1SD). This has two benefits. First, it makes main effect coefficients directly

247   interpretable even in the presence of interactions (Schielzeth, 2010). Second, for categorical

248   variables, having the intercept on an "average" rather than on one arbitrary default category avoids

249   the problem of putting a more precise prior on an arbitrary reference category (which would be

250   defined by the intercept only) than on the others (which would be defined by the intercept and one

251   or several other coefficients)(McElreath, 2020). Morph-specific coefficients (intercepts, slopes)

252   remain easy to obtain post-fitting, by simply adding the relevant posterior coefficients. Random

253   effects included box-level and individual-level intercepts as well as, in the case of exploration, the

254   associated slopes for temperature. This allowed us to estimate among-box and among-individual

255   variation in mean behavior and thermal behavioral reaction norms as well as the box- and individual-

256   level covariances among them (Dingemanse & Dochtermann, 2013).

257   We used a Normal(μ = ln(400), σ = 0.5) prior for the fixed effects intercepts (mean log- latencies), so

258   that ~99% of the probability mass was within the range of latencies that was observable during the

259   experiment (i.e. 0 to 1200 sec, see above), but not excluding larger values, because of censoring. We

260   set the other priors to be weakly informative and follow some suggestions by McElreath (2020): a

261   Normal(0,1) prior for the other fixed effects, a half-Normal(0, 1) prior for both random effect and

262   distributional standard deviations. For the random effects correlation matrices, we use an LKJ(η = 3)

263   prior, as it helps reach convergence faster than McElreath (2020)'s η = 2 default. Note that our choice

264   here is more skeptical of high correlations and thus penalizes against our hypotheses of interest

265   (there are detectable correlations).

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266   We partitioned total phenotypic variation V_P for each behavior into the following components: V_P =

267    $V_F + V_I + V_B + V_D$, where V_F is the fixed effect variation, including $V_{F(state)}$ the portion of fixed-effect

268   variance attributable to known individual state (banding pattern, environment of origin), i.e.

269   excluding experimental effects (test order, temperature) (estimated following de Villemereuil,

270   Morrissey, Nakagawa, & Schielzeth, 2018); V_I the average among-individual variation (including the

271   effect of random temperature slope, estimated following Johnson, 2014), with $V_{I(intercept)}$ the among-

272   individual variation at the average test temperature ($V_I = V_{I(intercept)}$ for boldness); V_B and $V_{B(intercept)}$ are

273   the equivalent box-level variances; and V_D is the distributional, or residual, variation. As pointed by

274   Wilson (2018) and de Villemereuil et al. (2018), there is in most cases no one "true" repeatability

275   estimate just as there is no one "true" way of partitioning the phenotypic variance pie; several

276   estimates with differing interpretations can be presented. Therefore, both absolute variance

277   components and analytical choices regarding repeatabilities should be made explicit. We estimated

278   the following two unadjusted repeatabilities (i.e. including the entirety of V_P in the denominator;

279   Nakagawa & Schielzeth, 2010): within-state repeatability $R_{(within-state)} = V_{I(intercept)} / V_P$, and what we

280   term total repeatability, $R_{(total)} = (V_{I(intercept)} + V_{F(state)})/V_P$. The proportion of persistent among-

281   individual variation that is attributable to individual state (banding and landscape of origin) is then

282   denoted by $V_{F(state)} / (V_{I(intercept)} + V_{F(state)})$. Variance components and repeatabilities are presented on

283   the observed data scale (sensu de Villemereuil, Schielzeth, Nakagawa, & Morrissey, 2016). Variance

284   components on the latent log scale (i.e. directly using model coefficients) led to qualitatively and

285   quantitatively similar results.

286   We ran four chains for 12000 iterations, with the first 2000 iterations of each chain used for warmup.

287   We checked mixing graphically and confirmed chain convergence using the improved $\hat{R}$ statistic by

288   Vehtari et al. (2020). The chains were run longer than the default number of iterations to ensure the

289   effective sample size was satisfactory for all parameters (both bulk- and tail-effective sample sizes

290   sensu Vehtari et al., 2020 at least > 400, here > 1000). All posterior summaries are given as mean

291   [95% highest posterior density interval].

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292   **Results**

293   Exploration was related to shell morph (Table 1, Fig. 2), with morph-specific intercepts, i.e. mean log-

294   latencies, for 0, 3 and 5-banded snails of 6.65 [6.59, 6.69], 6.64 [6.59, 6.69] and 6.71 [6.66, 6.76],

295   respectively. Five-banded snails were on average slower to explore their surroundings than either

296   three-banded or unbanded snails (in both cases, mean difference: 0.07 [0.01, 0.13])(Fig.2) Snails also

297   became slower as tests went on (Table 1). We found no clear evidence of an effect of the landscape

298   of origin on exploration. Snails explored faster with increasing temperature (Table 1, Fig. 2;

299   temperature slopes for 0, 3, and 5-banded snails: -0.12 [-0.16,-0.07], -0.11 [-0.16, -0.07], -0.10 [-

300   0.15,-0.06]). There was however no clear evidence that the slope of the temperature reaction norm

301   varied between the three morphs, or between snails coming from different landscapes (Table 1;

302   credible intervals for all interactions largely overlap 0).

303   Morphs also varied in average boldness (Table 1, Fig. 2), with morph-specific intercepts for 0, 3 and

304   5-banded snails of 2.84 [2.65, 3.04], 2.99 [2.79, 3.18] and 3.10 [2.90, 3.30], respectively. Unbanded

305   snails were bolder than five-banded snails (mean difference: -0.26 [-0.47, -0.05]); three-banded snails

306   presenting intermediate values, with no clear difference with either extreme morph. Again, there

307   was no evidence for landscape or landscape × morph effects.

308   Both exploration and boldness were repeatable at the individual level, with average repeatabilities in

309   the same range for both behaviors (Table 2). Including fixed effect variation due to individual state

310   (morph and landscape of origin) in the calculation only slightly increased repeatabilities. Indeed, the

311   proportion of persistent among-individual variation attributable to fixed effects was different from

312   zero but small, with over 90% of individual-level variation attributable to other, unmeasured sources

313   (Fig. 3, Table 2). Among-individual variation in temperature slopes was minimal, with variation in

314   intercepts explaining 98% [89%, 100%] of the average exploration V_I (Fig. 3, Table 2). Accordingly, we

315   find no clear evidence that the level of among-individual variation changes across the temperature

316   gradient; following equations in Brommer (2013), the ratio between latent-scale V_I at the lowest and

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317   highest tested temperatures is not different from 1 (0.76 [0.39, 1.13]). We also found no evidence of

318   widespread rank switching across the temperature gradient (faster than average individuals in one

319   environment remained overall faster across contexts): indeed, the cross-environmental correlation,

320   which is higher the more predicted individual rankings stay consistent across environmental

321   gradients (Brommer, 2013), was close to 1 when comparing the two extremes of the thermal

322   gradient (0.85 [0.62, 1.00] on the latent scale).

323   Variation among boxes was small but non-negligible, in the same range as the proportion of variation

324   explained by fixed effects for both behaviors. Average exploration and boldness were positively

325   correlated at the individual level (Table 3, Fig. 4). There was no evidence that among-individual

326   variation in responses to temperature was correlated with either mean exploration times or mean

327   boldness (Table 3). There was no evidence for box-level correlations among traits (Table 3).

328   **Discussion**

329   By showing that behaviors linked to boldness and exploration are repeatable in *Cepaea nemoralis*,

330   we add to a growing list of personality studies in gastropods, highlighting the usefulness of this taxon

331   to address key questions in behavioral ecology (see e.g. Ahlgren et al., 2015; Cornwell, McCarthy, &

332   Biro, 2020; Cornwell, McCarthy, Snyder, & Biro, 2019; Dahirel et al., 2017; Goodchild, Schmidt, &

333   DuRant, 2020; Seaman & Briffa, 2015). We note however that this list is biased towards freshwater

334   and marine gastropods; more studies are needed to understand among-individual variation in

335   behavior in land mollusks. Additionally, we demonstrated that boldness and exploration are

336   positively correlated in a common syndrome and that their expression varies depending on shell

337   banding, a trait under strictly genetic determinism (little to no plasticity) that has been the focus of a

338   lot of research in this species (Ożgo, 2009; Richards et al., 2013). Given how behavior can shape

339   effective thermal tolerance (Abram et al., 2017) or vulnerability to predation (e.g. Hulthén et al.,

340   2017), we believe these behavioral differences must be taken into account when discussing the

341   evolution of shell color in this model species.

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342   Unbanded snails were both bolder and explored faster than five-banded snails (Table 1, Fig. 2).

343   Three-banded snails behaved similarly to unbanded snails for exploration (but were intermediate

344   between unbanded and five-banded snails for boldness). This shows the "effectively unbanded"

345   category sometimes used in *Cepaea* studies (Cain & Sheppard, 1954; Ożgo & Schilthuizen, 2012) has

346   at least some behavioral relevance (that category groups together snails with little to no banding on

347   the side of their shell exposed to the sun). Exploration and boldness were positively correlated both

348   at the among-individual (Table 2) and among-morph levels (the shyest morph was also the slowest,

349   Fig. 2). At the individual level, while some clutches were laid during the experiments, we were not

350   able to test if this behavioral syndrome was integrated into a broader pace of life syndrome *sensu*

351   Réale et al. (2010) by linking behavioral and life-history variation. Indeed, we were unable to

352   ascertain the maternal and especially paternal origin of most clutches, and were not able to follow

353   snail fecundity or longevity over their entire life. There are however some indications in the literature

354   that more active/mobile snails are faster-growing (Oosterhoff, 1977), as the pace-of-life syndrome

355   hypothesis would predict.

356   Five-banded snails were on average shyer than unbanded snails (Fig. 2). Birds, thrushes in particular

357   (genus *Turdus*), are key predators of *Cepaea nemoralis* (Rosin, Lesicki, Kwieciński, Skórka, &

358   Tryjanowski, 2017; Rosin, Olborska, Surmacki, & Tryjanowski, 2011). Historically, both frequency-

359   dependent predation and direct visual selection due to crypsis have been invoked as explanations for

360   predator-dependent morph variation in *Cepaea* (Jones et al., 1977; Ożgo, 2009), but discussions

361   often used human vision as a baseline. More recently, crypsis explanations have received increased

362   support from an experiment using models of avian vision to more rigorously test how thrushes see

363   different shell morphs (Surmacki et al., 2013). In both our test sites, the boldest morph (unbanded

364   shell) is the least conspicuous (based on Surmacki et al., 2013), not the rarest. Building on the

365   phenotypic compensation hypothesis (i.e. that risk-taking individuals should be better defended;

366   Ahlgren et al., 2015; Kuo, Irschick, & Lailvaux, 2015), this result then adds support to crypsis-based

367   explanations of *Cepaea* morph variation. However, phenotypic compensation is not a hard rule, and

368   risk-taking individuals are sometimes less defended than risk-avoiding ones (De Winter et al., 2016;

369   Goodchild et al., 2020). Besides, snails are also predated by rodents (Rosin et al., 2011), and shell

370   morphs differ in shell strength in ways that go counter to the phenotypic compensation hypothesis

371   (5-banded shells being stronger; Rosin et al., 2013). The combined effect of color and shell

372   thickness/strength on predation risk remains to be studied. Finally, we must remember that (i) our

373   knowledge of how avian predators perceive snails is very limited (Surmacki et al., 2013), (ii) we only

374   tested a small set of the available morphs, which do not include the rarest background colors (pink

375   and brown), and (iii) shell banding is a trait under multiple selection pressures, including thermal

376   selection (see below).

377   Exploration speed was temperature-dependent: as expected from an ectothermic species, snails

378   were on average faster at higher temperatures (Fig. 2). The temperature reaction norm of

379   exploration was remarkably conserved among individuals (the near-totality of the among-individual

380   variance V_I was due to differences in average behavior, rather than in temperature slopes; Table 2,

381   Fig. 3). In addition, there was surprisingly no evidence that behavioral differences among morphs are

382   influenced by the thermal environment, whether we consider the environment of origin (no habitat ×

383   morph interaction) or the current environment (no effect of morph identity on thermal reaction

384   norms) (Table 1). This is despite abundant evidence in the literature for thermal selection on shell

385   morphs, based on both field comparisons (e.g. Richardson, 1974; Schilthuizen, 2013; Kerstes et al.,

386   2019; for this study, see Methods), and experiments (Lamotte, 1959; Tilling, 1983; Wolda, 1967).

387   Studies giving snails a choice between multiple temperatures show snail morphs do have different

388   thermal preferences that align with expectations based on thermal selection (Kavaliers, 1992). Some

389   studies suggest that snails use shade and humidity just as much (and potentially more) as

390   temperature as cues to adjust their behavior to microclimate (Ożgo & Kubea, 2005; Rosin et al.,

391   2018). Our exploration tests were short, under standardized lighting conditions and with no water,

392   and snails were brought back to favorable humidity soon after. It is possible longer experiments, or

393   experiments comparing the responses of snails from different habitats to realistic climate variation

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394 (including shade and/or humidity) would yield different responses. Maybe more importantly, we only

395 tested temperatures favorable for activity, i.e. the limited part of the thermal niche closer to the

396 optimum. Morph differences in behavior might be stronger closer to critical minimal or maximal

397 temperature thresholds (Tilling, 1983). This can be investigated by using a wider range of

398 temperatures and expanding the reaction norm approach used here to either a character state

399 approach (e.g. Houslay, Earley, Young, & Wilson, 2019) or a non-linear reaction norm approach

400 (Arnold, Kruuk, & Nicotra, 2019); both would account for the typical non-linearity of complete

401 thermal performance curves (Arnold et al., 2019). It is very important to note, however, that these

402 results do not mean populations from landscapes differing in sun exposure are identical in behavior,

403 even for the range of situations we tested. Indeed, because morphs differ in their behavior, and

404 because morph frequencies differ among landscapes (see Supplementary Material S1), the average

405 snail from a sun-exposed population may well be bolder and more active than its counterpart from a

406 shaded population.

407 In any case, the links between behaviors and morphs we observed are conserved across contexts,

408 despite (apparent) selection on shell morph. While this is not a definite proof by itself, we consider

409 this a first hint in favor of a genetic association between morphs and behaviors that cannot be easily

410 broken by environmental changes. In addition to studies aiming to confirm these behavioral traits are

411 heritable, further research into the physiological underpinnings of behavioral differences between

412 morphs (building on e.g. Kavaliers, 1992) and of shell color and pattern determination (Kerkvliet et

413 al., 2017) should help confirm (or infirm) this putative genetic correlation and elucidate its proximate

414 basis.

415 Assuming this genetic link is confirmed, any discussion about how selection on morph may influence

416 the evolution of behavior (or vice versa) must be tempered by one fact: the greater part of the

417 repeatable among-individual variation in behavior was not explained by shell morph (see Fig. 3, Table

418 1, and the fact that morph differences are hard to see from raw data in Fig. 2). It is in a way

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419   unsurprising, as we did not expect a single discrete trait to entirely constrain individual behavioral

420   variation. Indeed, the expression of animal personalities can be influenced by many unobserved

421   drivers and state variables which should have a priori limited links to shell morph and its drivers

422   (Burns et al., 2012; Petelle, Martin, & Blumstein, 2019; Sih et al., 2015; Wright et al., 2019). This

423   includes for instance sex or reproductive history (DiRienzo & Aonuma, 2017; Kralj-Fišer, Hebets, &

424   Kuntner, 2017), predation risk (Goodchild et al., 2020), age or life stage (Dahirel et al., 2017), or body

425   size (Santostefano et al., 2017). Snail behavior is particularly sensitive to population density including

426   during development (Cameron & Carter, 1979; Oosterhoff, 1977), an environmental axis we ignored

427   in the present study. Also, our study focused on relatively short-term repeatability; it is possible that

428   over larger time scales, the variance component related to morph differences plays a more

429   important role. In a fish community, for instance, some differences among species are detectable

430   over long but not short time scales (Harrison et al., 2019). Finally, some level of stochastic behavioral

431   individuality is inevitable even in the total absence of meaningful genetic and environmental

432   variation (Bierbach, Laskowski, & Wolf, 2017). What must be noted, though, is that some of this

433   "remaining" among-individual variation may still, actually, relate to shell morph. Indeed, because of

434   dominance within loci and especially epistatic relationships among loci, individuals that share the

435   same shell phenotype may actually vary greatly in terms of underlying shell genotype (e.g. having

436   one dominant allele at the « band presence » locus leads to total band absence and masks variation

437   at all other banding genes; Jones et al., 1977). However, while much is known about among-morph

438   variation in thermal tolerance, life history, physiology (Kavaliers, 1992; Kerstes et al., 2019; Lamotte,

439   1959; Oosterhoff, 1977; Richardson, 1974; Tilling, 1983; Wolda, 1967), we know nothing, as far as we

440   can tell, about within-morph, but among-genotype variation. Investigations using individuals of

441   known genotype obtained through repeated crosses or, as our knowledge of the actual molecular

442   underpinnings increases, through direct genotyping (Gonzalez, Aramendia, & Davison, 2019;

443   Kerkvliet et al., 2017), may shed light on this "hidden" genetic variation and whether it contributes to

444   the persistence of morph-related behavior differences.

18

445   Increased boldness and exploration have been tied to a higher probability of dispersal in many

446   species (Cote, Clobert, Brodin, Fogarty, & Sih, 2010), including land snails (Dahirel et al., 2017), and

447   non-random dispersal is now acknowledged as a potentially widespread force behind population

448   phenotypic divergence (Edelaar & Bolnick, 2012; Jacob, Bestion, Legrand, Clobert, & Cote, 2015).

449   Bolder animals are often thought to trade increased success against a greater predation risk (Hulthén

450   et al., 2017; but see Moiron, Laskowski, & Niemelä, 2020); predation is generally considered a key

451   driver of morphological differences in *Cepaea*, and plays a key role in dispersal across taxa (Fronhofer

452   et al., 2018). Although active dispersal can safely be dismissed as a driver of continental-scale

453   differences in morph frequencies, our results point to *Cepaea* as a good model to understand how

454   existing behavioral differences may drive local-scale morphological differences (and vice versa). By

455   building on, and complementing, a decades-long history of evolutionary research, this will help us

456   better understand the role of behavior, and constraints on behavioral variation, in shaping responses

457   to rapid environmental changes (Candolin & Wong, 2012), including landscape alteration and climate

458   change.

459   **References**

460   Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on

461   ectothermic animals: Unifying thermal physiology and behavioural plasticity. *Biological*

462   *Reviews*, *92*(4), 1859–1876. doi: 10.1111/brv.12312

463   Ahlgren, J., Chapman, B. B., Nilsson, P. A., & Brönmark, C. (2015). Individual boldness is linked to

464   protective shell shape in aquatic snails. *Biology Letters*, *11*(4), 20150029. doi:

465   10.1098/rsbl.2015.0029

466   Arnold, P. A., Kruuk, L. E. B., & Nicotra, A. B. (2019). How to analyse plant phenotypic plasticity in

467   response to a changing climate. *New Phytologist*, *222*(3), 1235–1241. doi:

468   10.1111/nph.15656

19

469   ASAB, & ABS. (2020). Guidelines for the treatment of animals in behavioural research and teaching.

470   *Animal Behaviour*, *159*, I‐XI. doi: 10.1016/j.anbehav.2019.11.002

471   Bierbach, D., Laskowski, K. L., & Wolf, M. (2017). Behavioural individuality in clonal fish arises despite

472   near-identical rearing conditions. *Nature Communications*, *8*, 15361. doi:

473   10.1038/ncomms15361

474   Brommer, J. E. (2013). Variation in plasticity of personality traits implies that the ranking of

475   personality measures changes between environmental contexts‐: Calculating the cross-

476   environmental correlation. *Behavioral Ecology and Sociobiology*, *67*(10), 1709‐1718. doi:

477   10.1007/s00265-013-1603-9

478   Bürkner, P.-C. (2017). brms‐: An R package for Bayesian multilevel models using Stan. *Journal of*

479   *Statistical Software*, *80*(1), 1‐28. doi: 10.18637/jss.v080.i01

480   Burns, J. G., Svetec, N., Rowe, L., Mery, F., Dolan, M. J., Boyce, W. T., & Sokolowski, M. B. (2012).

481   Gene–environment interplay in *Drosophila melanogaster*‐: Chronic food deprivation in early

482   life affects adult exploratory and fitness traits. *Proceedings of the National Academy of*

483   *Sciences of the United States of America*, *109*(Suppl 2), 17239‐17244. doi:

484   10.1073/pnas.1121265109

485   Cain, A. J., & Sheppard, P. M. (1954). Natural selection in *Cepaea*. *Genetics*, *39*(1), 89‐116.

486   Cameron, R. A. D. (1970). The effect of temperature on the activity of three species of helicid snail

487   (Mollusca‐: Gastropoda). *Journal of Zoology*, *162*(3), 303‐315. doi: 10.1111/j.1469-

488   7998.1970.tb01267.x

489   Cameron, R. A. D., & Carter, M. A. (1979). Intra- and interspecific effects of population density on

490   growth and activity in some helicid land snails (Gastropoda‐: Pulmonata). *Journal of Animal*

491   *Ecology*, *48*(1), 237‐246. doi: 10.2307/4111

492   Candolin, U., & Wong, B. B. M. (Éds.). (2012). *Behavioural responses to a changing world‐:*

493   *Mechanisms and consequences* (1st edition). Oxford: Oxford University Press.

20

494   Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., … Riddell, A. (2017).

495   Stan: A probabilistic programming language. *Journal of Statistical Software*, *76*(1), 132.

496   doi: 10.18637/jss.v076.i01

497   Chang, H.-W. (1991). Activity and weight loss in relation to solar radiation in the polymorphic land

498   snail *Cepaea nemoralis*. *Journal of Zoology*, *225*(2), 213225. doi: 10.1111/j.1469-

499   7998.1991.tb03812.x

500   Cloyed, C. S., Dell, A. I., Hayes, T., Kordas, R. L., & O'Gorman, E. J. (2019). Long-term exposure to

501   higher temperature increases the thermal sensitivity of grazer metabolism and movement.

502   *Journal of Animal Ecology*, *0*(0). doi: 10.1111/1365-2656.12976

503   Cook, L. M. (2008). Variation with habitat in *Cepaea nemoralis*: The Cain & Sheppard diagram.

504   *Journal of Molluscan Studies*, *74*(3), 239243. doi: 10.1093/mollus/eyn011

505   Cornwell, T. O., McCarthy, I. D., & Biro, P. A. (2020). Integration of physiology, behaviour and life

506   history traits: Personality and pace of life in a marine gastropod. *Animal Behaviour*, *163*,

507   155162. doi: 10.1016/j.anbehav.2020.03.009

508   Cornwell, T. O., McCarthy, I. D., Snyder, C. R. A., & Biro, P. A. (2019). The influence of environmental

509   gradients on individual behaviour: Individual plasticity is consistent across risk and

510   temperature gradients. *Journal of Animal Ecology*, *88*(4), 511520. doi: 10.1111/1365-

511   2656.12935

512   Cote, J., Clobert, J., Brodin, T., Fogarty, S., & Sih, A. (2010). Personality-dependent dispersal:

513   Characterization, ontogeny and consequences for spatially structured populations.

514   *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*(1560),

515   40654076. doi: 10.1098/rstb.2010.0176

516   Dahirel, M., Vong, A., Ansart, A., & Madec, L. (2017). Individual boldness is life stage-dependent and

517   linked to dispersal in a hermaphrodite land snail. *Ecological Research*, *32*, 751755. doi:

518   10.1007/s11284-017-1484-x

21

519   Davison, A., Jackson, H. J., Murphy, E. W., & Reader, T. (2019). Discrete or indiscrete? Redefining the

520   colour polymorphism of the land snail *Cepaea nemoralis*. *Heredity*, 1. doi: 10.1038/s41437-

521   019-0189-z

522   De Winter, G., Ramalho Martins, H., Trovo, R. A., & Chapman, B. B. (2016). Knights in shining armour

523   are not necessarily bold : Defensive morphology correlates negatively with boldness, but

524   positively with activity, in wild threespine stickleback, *Gasterosteus aculeatus*. *Evolutionary*

525   *Ecology Research*, *17*, 1 12.

526   Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour :

527   Mixed-effect modelling approaches. *Journal of Animal Ecology*, *82*(1), 39 54. doi:

528   10.1111/1365-2656.12013

529   Dingemanse, N. J., Kazem, A. J., Réale, D., & Wright, J. (2010). Behavioural reaction norms : Animal

530   personality meets individual plasticity. *Trends in Ecology & Evolution*, *25*(2), 81 89. doi:

531   10.1016/j.tree.2009.07.013

532   Dingemanse, N. J., & Wright, J. (2020). Criteria for acceptable studies of animal personality and

533   behavioural syndromes. *Ethology*, *126*(9), 865 869. doi: 10.1111/eth.13082

534   DiRienzo, N., & Aonuma, H. (2017). Individual differences are consistent across changes in mating

535   status and mediated by biogenic amines. *Behavioral Ecology and Sociobiology*, *71*(8), 118.

536   doi: 10.1007/s00265-017-2345-x

537   Dochtermann, N. A., & Dingemanse, N. J. (2013). Behavioral syndromes as evolutionary constraints.

538   *Behavioral Ecology*, *24*(4), 806 811. doi: 10.1093/beheco/art002

539   Edelaar, P., & Bolnick, D. I. (2012). Non-random gene flow : An underappreciated force in evolution

540   and ecology. *Trends in Ecology & Evolution*, *27*(12), 659 665. doi:

541   10.1016/j.tree.2012.07.009

542   Fronhofer, E. A., Legrand, D., Altermatt, F., Ansart, A., Blanchet, S., Bonte, D., … Cote, J. (2018).

543   Bottom-up and top-down control of dispersal across major organismal groups. *Nature*

544   *Ecology & Evolution*, *2*(12), 1859 1863. doi: 10.1038/s41559-018-0686-0

22

545   Gabry, J., Simpson, D., Vehtari, A., Betancourt, M., & Gelman, A. (2019). Visualization in Bayesian

546   workflow. *Journal of the Royal Statistical Society: Series A (Statistics in Society), 182*(2),

547   389⬚402. doi: 10.1111/rssa.12378

548   GBIF Secretariat. (2020). Cepaea nemoralis *(Linnaeus, 1758)* in *GBIF Backbone Taxonomy. Checklist*

549   *dataset accessed via GBIF.org on 2020-08-11*. Consulté à l'adresse

550   https://doi.org/10.15468/39omei

551   Gonzalez, D. R., Aramendia, A. C., & Davison, A. (2019). Recombination within the *Cepaea nemoralis*

552   supergene is confounded by incomplete penetrance and epistasis. *Heredity*, 1. doi:

553   10.1038/s41437-019-0190-6

554   Goodchild, C. G., Schmidt, L. M., & DuRant, S. E. (2020). Evidence for the 'behavioural character'

555   hypothesis⬚: Does boldness programme disparate antipredator strategies? *Animal*

556   *Behaviour, 164*, 123⬚132. doi: 10.1016/j.anbehav.2020.04.010

557   Harrison, P. M., Keeler, R. A., Robichaud, D., Mossop, B., Power, M., & Cooke, S. J. (2019). Individual

558   differences exceed species differences in the movements of a river fish community.

559   *Behavioral Ecology*, arz076. doi: 10.1093/beheco/arz076

560   Heath, D. J. (1975). Colour, sunlight and internal temperatures in the land-snail *Cepaea nemoralis*

561   (L.). *Oecologia, 19*(1), 29⬚38. doi: 10.1007/BF00377587

562   Henry, P.-Y., & Jarne, P. (2007). Marking hard-shelled gastropods⬚: Tag loss, impact on life-history

563   traits, and perspectives in biology. *Invertebrate Biology, 126*(2), 138⬚153. doi:

564   10.1111/j.1744-7410.2007.00084.x

565   Houslay, T. M., Earley, R. L., Young, A. J., & Wilson, A. J. (2019). Habituation and individual variation in

566   the endocrine stress response in the Trinidadian guppy (*Poecilia reticulata*). *General and*

567   *Comparative Endocrinology, 270*, 113⬚122. doi: 10.1016/j.ygcen.2018.10.013

568   Houslay, Thomas M., & Wilson, A. J. (2017). Avoiding the misuse of BLUP in behavioural ecology.

569   *Behavioral Ecology, 28*(4), 948⬚952. doi: 10.1093/beheco/arx023

23

570   Hulthén, K., Chapman, B. B., Nilsson, P. A., Hansson, L.-A., Skov, C., Brodersen, J., … Brönmark, C.

571   (2017). A predation cost to bold fish in the wild. *Scientific Reports*, *7*(1), 1239. doi:

572   10.1038/s41598-017-01270-w

573   Jacob, S., Bestion, E., Legrand, D., Clobert, J., & Cote, J. (2015). Habitat matching and spatial

574   heterogeneity of phenotypes : Implications for metapopulation and metacommunity

575   functioning. *Evolutionary Ecology*, *29*(6), 851 871. doi: 10.1007/s10682-015-9776-5

576   Johnson, P. C. D. (2014). Extension of Nakagawa & Schielzeth's R^2_{GLMM} to random slopes models.

577   *Methods in Ecology and Evolution*, *5*(9), 944 946. doi: 10.1111/2041-210X.12225

578   Jones, J. S. (1982). Genetic differences in individual behaviour associated with shell polymorphism in

579   the snail *Cepaea nemoralis*. *Nature*, *298*(5876), 749 750. doi: 10.1038/298749a0

580   Jones, J. S., Leith, B. H., & Rawlings, P. (1977). Polymorphism in *Cepaea* : A problem with too many

581   solutions? *Annual Review of Ecology and Systematics*, *8*, 109 143. JSTOR. Consulté à

582   l'adresse JSTOR.

583   Kavaliers, M. (1992). Opioid systems, behavioral thermoregulation and shell polymorphism in the

584   land snail, *Cepaea nemoralis*. *Journal of Comparative Physiology B*, *162*(2), 172 178. doi:

585   10.1007/BF00398344

586   Kay, M. (2019). *tidybayes : Tidy data and geoms for Bayesian models*. doi: 10.5281/zenodo.1308151

587   Kerkvliet, J., Boer, T. de, Schilthuizen, M., & Kraaijeveld, K. (2017). Candidate genes for shell colour

588   polymorphism in *Cepaea nemoralis*. *PeerJ*, *5*, e3715. doi: 10.7717/peerj.3715

589   Kern, E. M. A., Robinson, D., Gass, E., Godwin, J., & Langerhans, R. B. (2016). Correlated evolution of

590   personality, morphology and performance. *Animal Behaviour*, *117*, 79 86. doi:

591   10.1016/j.anbehav.2016.04.007

592   Kerstes, N. A. G., Breeschoten, T., Kalkman, V. J., & Schilthuizen, M. (2019). Snail shell colour

593   evolution in urban heat islands detected via citizen science. *Communications Biology*, *2*(1),

594   264. doi: 10.1038/s42003-019-0511-6

24

595   Kralj-Fišer, S., Hebets, E. A., & Kuntner, M. (2017). Different patterns of behavioral variation across

596   and within species of spiders with differing degrees of urbanization. *Behavioral Ecology and*

597   *Sociobiology*, *71*(8), 125. doi: 10.1007/s00265-017-2353-x

598   Kralj-Fišer, S., & Schuett, W. (2014). Studying personality variation in invertebrates: Why bother?

599   *Animal Behaviour*, *91*, 41–52. doi: 10.1016/j.anbehav.2014.02.016

600   Kuo, C.-Y., Irschick, D. J., & Lailvaux, S. P. (2015). Trait compensation between boldness and the

601   propensity for tail autotomy under different food availabilities in similarly aged brown anole

602   lizards. *Functional Ecology*, *29*(3), 385–392. doi: 10.1111/1365-2435.12324

603   Lamotte, M. (1959). Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring Harbor*

604   *Symposia on Quantitative Biology*, *24*(0), 65–86. doi: 10.1101/SQB.1959.024.01.009

605   McElreath, R. (2020). *Statistical rethinking: A Bayesian course with examples in R and Stan* (2nd

606   edition). Boca Raton, USA: Chapman and Hall/CRC.

607   Moiron, M., Laskowski, K. L., & Niemelä, P. T. (2020). Individual differences in behaviour explain

608   variation in survival: A meta-analysis. *Ecology Letters*, *23*(2), 399–408. doi:

609   10.1111/ele.13438

610   Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical

611   guide for biologists. *Biological Reviews*, *85*(4), 935–956. doi: 10.1111/j.1469-

612   185X.2010.00141.x

613   Niemelä, P. T., & Dingemanse, N. J. (2018). On the usage of single measurements in behavioural

614   ecology research on individual differences. *Animal Behaviour*, *145*, 99–105. doi:

615   10.1016/j.anbehav.2018.09.012

616   Oosterhoff, L. M. (1977). Variation in growth rate as an ecological factor in the landsnail *Cepaea*

617   *nemoralis* (L.). *Netherlands Journal of Zoology*, *27*(1), 1–132. doi: 10.1163/002829677X00072

618   Ożgo, M. (2009). Current problems in the research of *Cepaea* polymorphism. *Folia Malacologica*,

619   *16*(2), 55–60. doi: 10.12657/folmal.016.009

620   Ozgo, M., & Kinnison, M. T. (2008). Contingency and determinism during convergent contemporary

621   evolution in the polymorphic land snail, *Cepaea nemoralis. Evolutionary Ecology Research*,

622   *10*, 721–733.

623   Ożgo, M., & Kubea, A. (2005). Humidity and the effect of shell colour on activity of *Cepaea nemoralis*

624   (Linnaeus, 1758). *Folia Malacologica, 13*(3).

625   Ożgo, M., Liew, T.-S., Webster, N. B., & Schilthuizen, M. (2017). Inferring microevolution from

626   museum collections and resampling: Lessons learned from *Cepaea. PeerJ, 5*, e3938. doi:

627   10.7717/peerj.3938

628   Ożgo, M., & Schilthuizen, M. (2012). Evolutionary change in *Cepaea nemoralis* shell colour over 43

629   years. *Global Change Biology, 18*(1), 74–81. doi: 10.1111/j.1365-2486.2011.02514.x

630   Pedersen, T. L. (2019). *patchwork: The composer of plots*. Consulté à l'adresse https://CRAN.R-

631   project.org/package=patchwork

632   Petelle, M. B., Martin, J. G. A., & Blumstein, D. T. (2019). Mixed support for state maintaining risky

633   personality traits in yellow-bellied marmots. *Animal Behaviour, 150*, 177–188. doi:

634   10.1016/j.anbehav.2019.02.008

635   R Core Team. (2020). R: a language and environment for statistical computing (Version 4.0.0). Vienna,

636   Austria: R Foundation for Statistical Computing. Consulté à l'adresse https://www.R-

637   project.org/

638   Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P.-O. (2010).

639   Personality and the emergence of the pace-of-life syndrome concept at the population level.

640   *Philosophical Transactions of the Royal Society of London B: Biological Sciences, 365*(1560),

641   4051–4063. doi: 10.1098/rstb.2010.0208

642   Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal

643   temperament within ecology and evolution. *Biological Reviews, 82*(2), 291–318. doi:

644   10.1111/j.1469-185X.2007.00010.x

26

645   Richards, P. M., Liu, M. M., Lowe, N., Davey, J. W., Blaxter, M. L., & Davison, A. (2013). RAD-Seq

646   derived markers flank the shell colour and banding loci of the *Cepaea nemoralis* supergene.

647   *Molecular Ecology*, *22*(11), 3077–3089. doi: 10.1111/mec.12262

648   Richardson, A. M. M. (1974). Differential climatic selection in natural population of land snail *Cepaea*

649   *nemoralis*. *Nature*, *247*(5442), 572. doi: 10.1038/247572a0

650   Rosin, Z. M., Kobak, J., Lesicki, A., & Tryjanowski, P. (2013). Differential shell strength of *Cepaea*

651   *nemoralis* colour morphs—Implications for their anti-predator defence.

652   *Naturwissenschaften*, *100*(9), 843–851. doi: 10.1007/s00114-013-1084-8

653   Rosin, Z. M., Kwieciński, Z., Lesicki, A., Skórka, P., Kobak, J., Szymańska, A., … Tryjanowski, P. (2018).

654   Shell colour, temperature, (micro)habitat structure and predator pressure affect the

655   behaviour of *Cepaea nemoralis*. *The Science of Nature*, *105*(5), 35. doi: 10.1007/s00114-018-

656   1560-2

657   Rosin, Z. M., Lesicki, A., Kwieciński, Z., Skórka, P., & Tryjanowski, P. (2017). Land snails benefit from

658   human alterations in rural landscapes and habitats. *Ecosphere*, *8*(7), e01874. doi:

659   10.1002/ecs2.1874

660   Rosin, Z. M., Olborska, P., Surmacki, A., & Tryjanowski, P. (2011). Differences in predatory pressure

661   on terrestrial snails by birds and mammals. *Journal of Biosciences*, *36*(4), 691–699. doi:

662   10.1007/s12038-011-9077-2

663   Royauté, R., Berdal, M. A., Garrison, C. R., & Dochtermann, N. A. (2018). Paceless life? A meta-

664   analysis of the pace-of-life syndrome hypothesis. *Behavioral Ecology and Sociobiology*, *72*(3),

665   64. doi: 10.1007/s00265-018-2472-z

666   Santostefano, F., Wilson, A. J., Niemelä, P. T., & Dingemanse, N. J. (2017). Behavioural mediators of

667   genetic life-history trade-offs: A test of the pace-of-life syndrome hypothesis in field

668   crickets. *Proc. R. Soc. B*, *284*(1864), 20171567. doi: 10.1098/rspb.2017.1567

669   Schielzeth, H. (2010). Simple means to improve the interpretability of regression coefficients.

670   *Methods in Ecology and Evolution*, *1*(2), 103–113. doi: 10.1111/j.2041-210X.2010.00012.x

27

671   Schilthuizen, M. (2013). Rapid, habitat-related evolution of land snail colour morphs on reclaimed

672   land. *Heredity*, *110*(3), 247–252.

673   Seaman, B., & Briffa, M. (2015). Parasites and personality in periwinkles (*Littorina littorea*): Infection

674   status is associated with mean-level boldness but not repeatability. *Behavioural Processes*,

675   *115*, 132–134. doi: 10.1016/j.beproc.2015.03.014

676   Sih, A., Bell, A. M., Johnson, J. C., & Ziemba, R. E. (2004). Behavioral syndromes: An integrative

677   overview. *The Quarterly Review of Biology*, *79*(3), 241–277. doi: 10.1086/422893

678   Sih, A., Mathot, K. J., Moirón, M., Montiglio, P.-O., Wolf, M., & Dingemanse, N. J. (2015). Animal

679   personality and state–behaviour feedbacks: A review and guide for empiricists. *Trends in*

680   *Ecology & Evolution*, *30*(1), 50–60. doi: 10.1016/j.tree.2014.11.004

681   Silvertown, J., Cook, L., Cameron, R., Dodd, M., McConway, K., Worthington, J., … Juan, X. (2011).

682   Citizen science reveals unexpected continental-scale evolutionary change in a model

683   organism. *PLOS ONE*, *6*(4), e18927. doi: 10.1371/journal.pone.0018927

684   Surmacki, A., Ożarowska-Nowicka, A., & Rosin, Z. M. (2013). Color polymorphism in a land snail

685   *Cepaea nemoralis* (Pulmonata: Helicidae) as viewed by potential avian predators. *Die*

686   *Naturwissenschaften*, *100*(6), 533–540. doi: 10.1007/s00114-013-1049-y

687   Tilling, S. M. (1983). An experimental investigation of the behaviour and mortality of artificial and

688   natural morphs of *Cepaea nemoralis* (L.). *Biological Journal of the Linnean Society*, *19*(1),

689   35–50. doi: 10.1111/j.1095-8312.1983.tb00775.x

690   Vehtari, A., Gelman, A., Simpson, D., Carpenter, B., & Bürkner, P.-C. (2020). Rank-normalization,

691   folding, and localization: An improved \widehat{R} for assessing convergence of MCMC.

692   *Bayesian Analysis*. doi: 10.1214/20-BA1221

693   Villemereuil, P. de, Morrissey, M. B., Nakagawa, S., & Schielzeth, H. (2018). Fixed-effect variance and

694   the estimation of repeatabilities and heritabilities: Issues and solutions. *Journal of*

695   *Evolutionary Biology*, *31*(4), 621–632. doi: 10.1111/jeb.13232

28

696   Villemereuil, Pierre de, Schielzeth, H., Nakagawa, S., & Morrissey, M. (2016). General methods for

697   evolutionary quantitative genetic inference from Generalized Mixed Models. *Genetics*,

698   *204*(3), 1281–1294. doi: 10.1534/genetics.115.186536

699   Webster, M. M., & Rutz, C. (2020). How STRANGE are your study animals? *Nature, 582*(7812),

700   337–340. doi: 10.1038/d41586-020-01751-5

701   Welter-Schultes, F. (2012). *European non-marine molluscs, a guide for species identification*.

702   Göttingen: Planet Poster Editions.

703   Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., … Yutani, H. (2019).

704   Welcome to the Tidyverse. *Journal of Open Source Software, 4*(43), 1686. doi:

705   10.21105/joss.01686

706   Wilson, A. J. (2018). How should we interpret estimates of individual repeatability? *Evolution Letters*,

707   *2*(1), 4–8. doi: 10.1002/evl3.40

708   Wolda, H. (1967). The effect of temperature on reproduction in some morphs of the landsnail

709   *Cepaea nemoralis* (L.). *Evolution, 21*(1), 117–129. doi: 10.1111/j.1558-5646.1967.tb00135.x

710   Wolf, M., & McNamara, J. M. (2012). On the evolution of personalities via frequency-dependent

711   selection. *The American Naturalist, 179*(6), 679–692. doi: 10.1086/665656

712   Wolf, M., & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences.

713   *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1560), 3959–3968.

714   doi: 10.1098/rstb.2010.0215

715   Wright, J., Bolstad, G. H., Araya-Ajoy, Y. G., & Dingemanse, N. J. (2019). Life-history evolution under

716   fluctuating density-dependent selection and the adaptive alignment of pace-of-life

717   syndromes. *Biological Reviews, 94*(1), 230–247. doi: 10.1111/brv.12451

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720   **Tables**

721   **Table 1:** Estimated fixed effect parameters of the model explaining exploration and boldness

722   latencies (mean and 95% credible intervals). Continuous explanatory variables are centered and

723   scaled, and categorical variables converted to centered dummy variables; the intercept then refers to

724   the behavior of a hypothetical "average" snail.

	Mean effect (95% credible interval)	
	Exploration	Boldness
Fixed effects: main effects		
Intercept (log-latency of an "average" individual)	6.67 (6.63 ; 6.70)	2.98 (2.83 ; 3.13)
banding = 3 bands	-0.00 (-0.06 ; 0.06)	0.15 (-0.06 ; 0.36)
banding = 5 bands	**0.07 (0.01 ; 0.13)**	**0.26 (0.05 ; 0.47)**
landscape of origin = shaded	0.06 (-0.02 ; 0.13)	0.20 (-0.10 ; 0.50)
test temperature	**-0.11 (-0.15 ; -0.07)**	--
test order	**0.04 (0.00 ; 0.07)**	0.08 (-0.04 ; 0.19)
Fixed effects: interactions		
banding = 3bands × landscape = shaded	-0.07 (-0.19 ; 0.05)	0.16 (-0.25 ; 0.56)
banding = 5bands × landscape = shaded	-0.01 (-0.13 ; 0.11)	0.24 (-0.16 ; 0.65)
banding = 3bands × temperature	0.00 (-0.04 ; 0.04)	--
banding = 5bands × temperature	0.01 (-0.03 ; 0.05)	--
landscape = shaded × temperature	-0.05 (-0.13 ; 0.03)	--
banding = 3bands × landscape = shaded × temperature	-0.02 (-0.10 ; 0.06)	--
banding = 5bands × landscape = shaded × temperature	0.02 (-0.06 ; 0.10)	--

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726   **Table 2.** Variance partitioning and repeatabilities. Estimated variances and 95% credible interval are

727   obtained from a multivariate mixed model. Variances are estimated on the observed scale *sensu* de

728   Villemereuil et al (2016). Variances are rounded to the nearest unit. Readers looking at the data may

729   note that the total variances V_P are higher than the empirically observed variances. This is because

730   the latter are underestimated, due to censoring.

	Posterior mean (95% credible interval)	
	Exploration	Boldness
Variance partitioning (observed scale, in sec²)		
Fixed effects V_F	13556 (6349 ; 21112)	49 (6 ; 110)
- explained by state	2185 (329 ; 4360)	46 (4 ; 105)
Among boxes V_B	8468 (2984 ; 15132)	128 (12; 307)
- random intercept only	3102 (0 ; 7101)	128 (12; 307)
Among individuals V_I	25677 (17966 ; 33628)	542 (263; 875)
- random intercept only	24346 (17322 ; 32035)	542 (263; 875)
Residual V_D	73976 (63439 ; 84520)	1795 (966 ; 2816)
Total variance V_P	121676 (102632 ; 141659)	2513 (1334 ; 3960)
Repeatabilities (observed scale)		
$R_{(within\text{-}state)}$	0.20 (0.15 ; 0.25)	0.22 (0.15 ; 0.29)
$R_{(total)}$	0.22 (0.17 ; 0.27)	0.24 (0.17 ; 0.31)
$R_{(within\text{-}state)}$ / $R_{(total)}$	0.92 (0.84 ; 0.98)	0.92 (0.84 ; 0.99)

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733   **Table 3.** Random effect correlation matrices (latent scale, mean and 95% credible intervals). Box-

734   level correlations are above the diagonal, individual-level correlations below the diagonal.

	Boldness, intercept	Exploration, intercept	Exploration, temperature slope
Boldness, intercept		0.12 (-0.42 ; 0.63)	0.38 (-0.05 ; 0.79)
Exploration, intercept	**0.28 (0.10 ; 0.46)**		-0.19 (-0.69 ; 0.33)
Exploration, temperature slope	-0.19 (-0.67 ; 0.32)	0.29 (-0.24 ; 0.76)	

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737 **Figure legends**

738 **Figure 1.** Study species and sites. (A) An unbanded yellow *Cepaea nemoralis*, showing the position of

739 individual paint marks on the shell (B) Representative shells of the three studied morphs as seen

740 from above: yellow unbanded, three-banded, and five-banded snails (C) Study sites location in

741 France; the open habitat (D) and the shaded habitat (E) are separated by about 2 km. Photographs
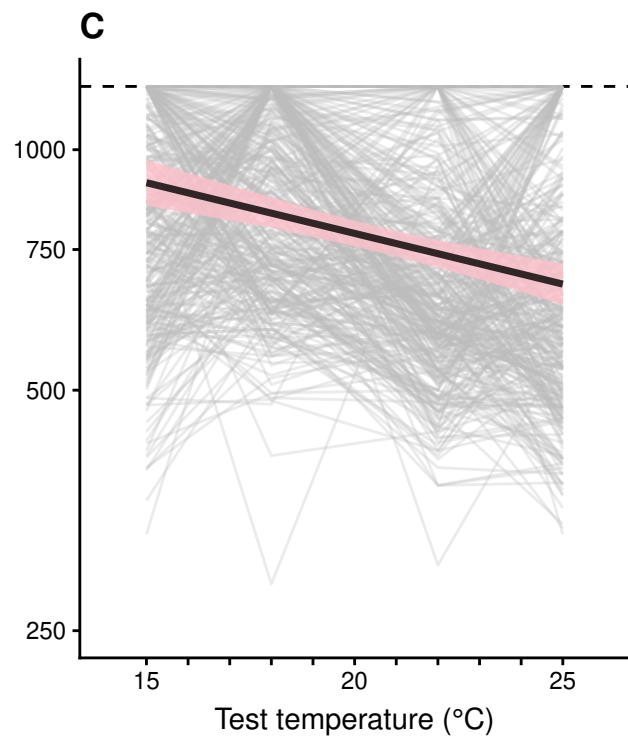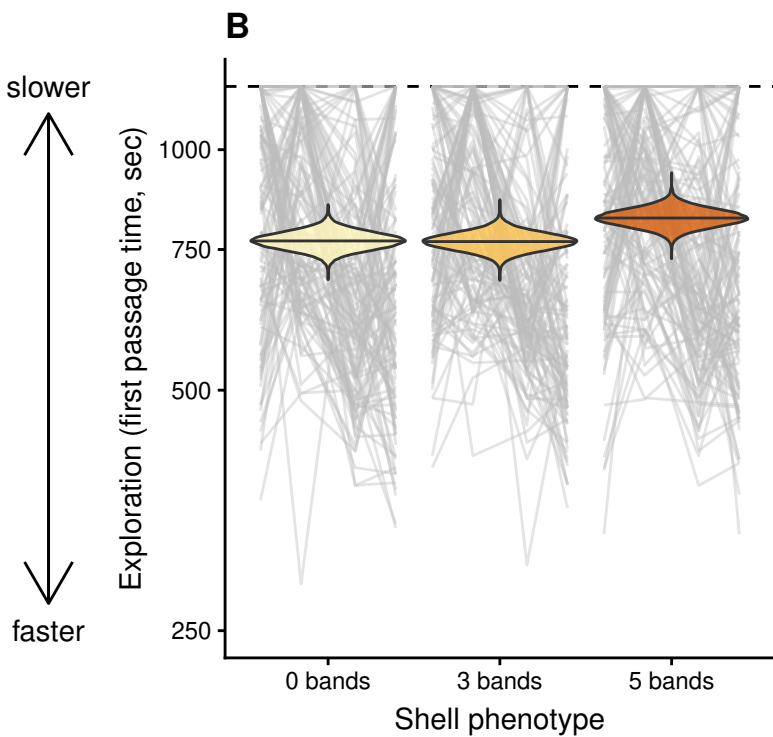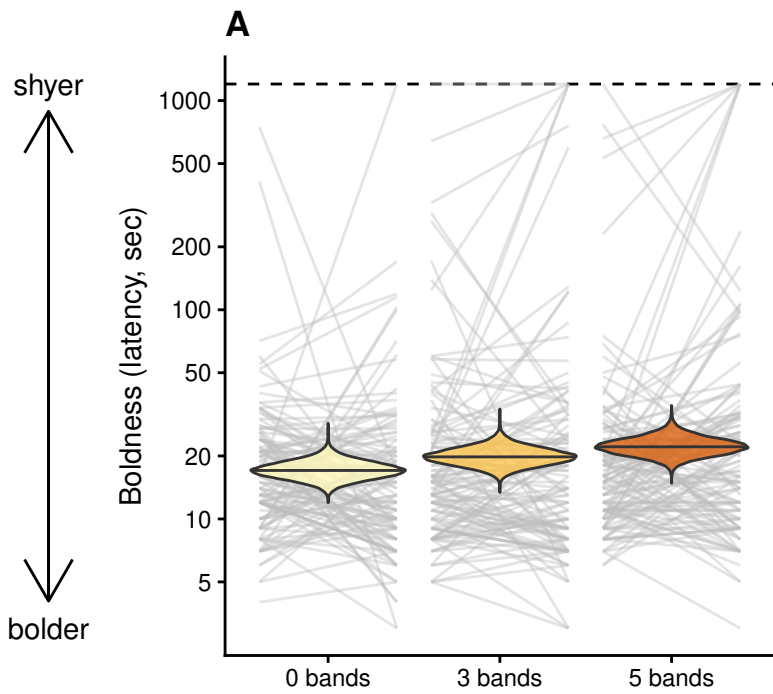
742 were taken during winter, when snails were collected.

743 **Figure 2.** (A; B) posterior distributions of median boldness (A) and exploration (B) in relation to shell

744 morph. The model estimates the mean log-latencies, which correspond to the medians on the

745 observed latencies scale. (C) Mean and 95% credible band for the relationship between exploration

746 latency and test temperature. Grey lines connect trials from the same individual. Values are plotted

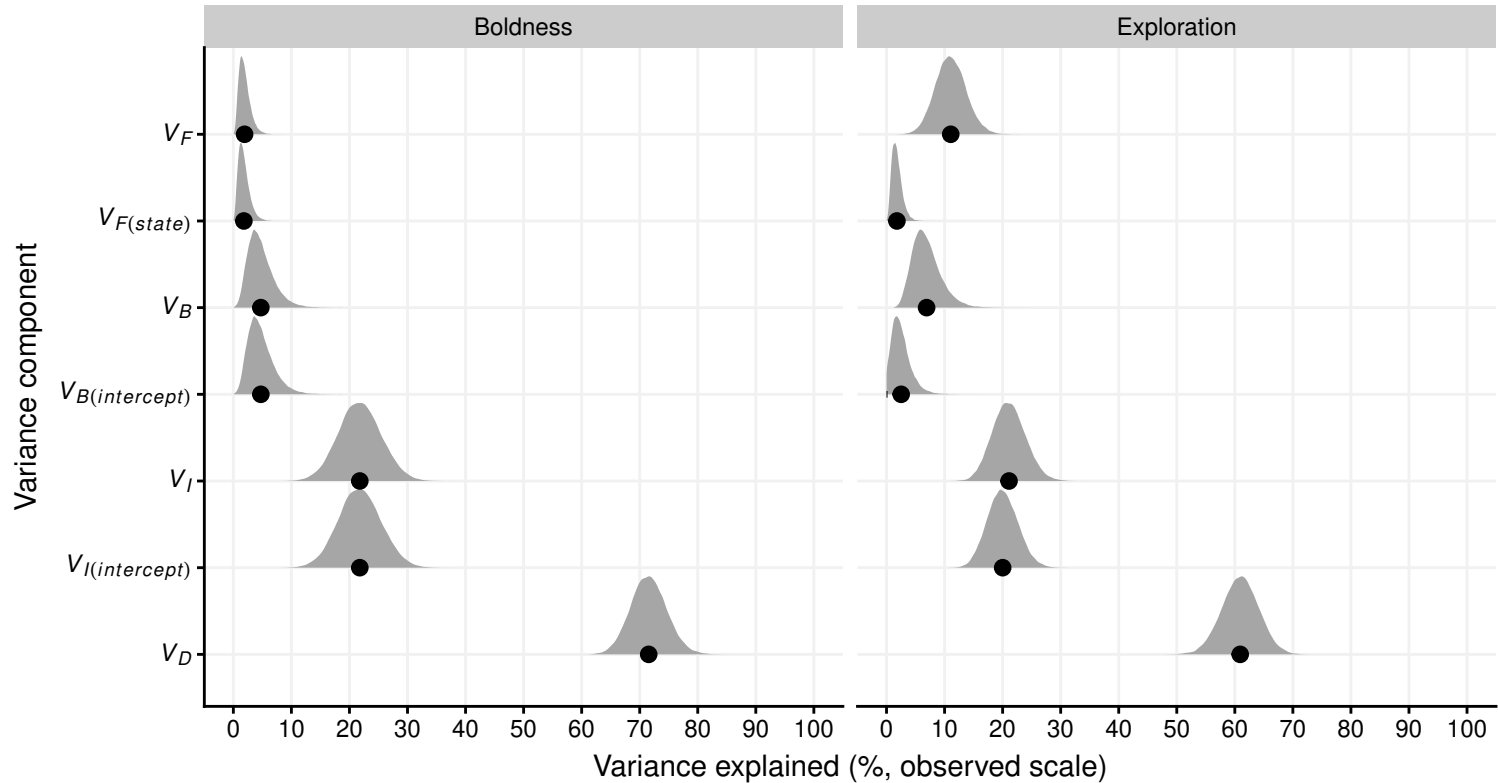747 on a log-transformed axis. N = 360 individuals (60 per landscape × morph combination)

748 **Figure 3.** Mean (points) and posteriors for the proportion of variance explained by the different

749 variance components. For boldness, V_B and $V_{B(intercept)}$ are exactly equal by definition (same for V_I and

750 $V_{I(intercept)}$); see Methods and Table 2 for details on this and the names of the variance components.
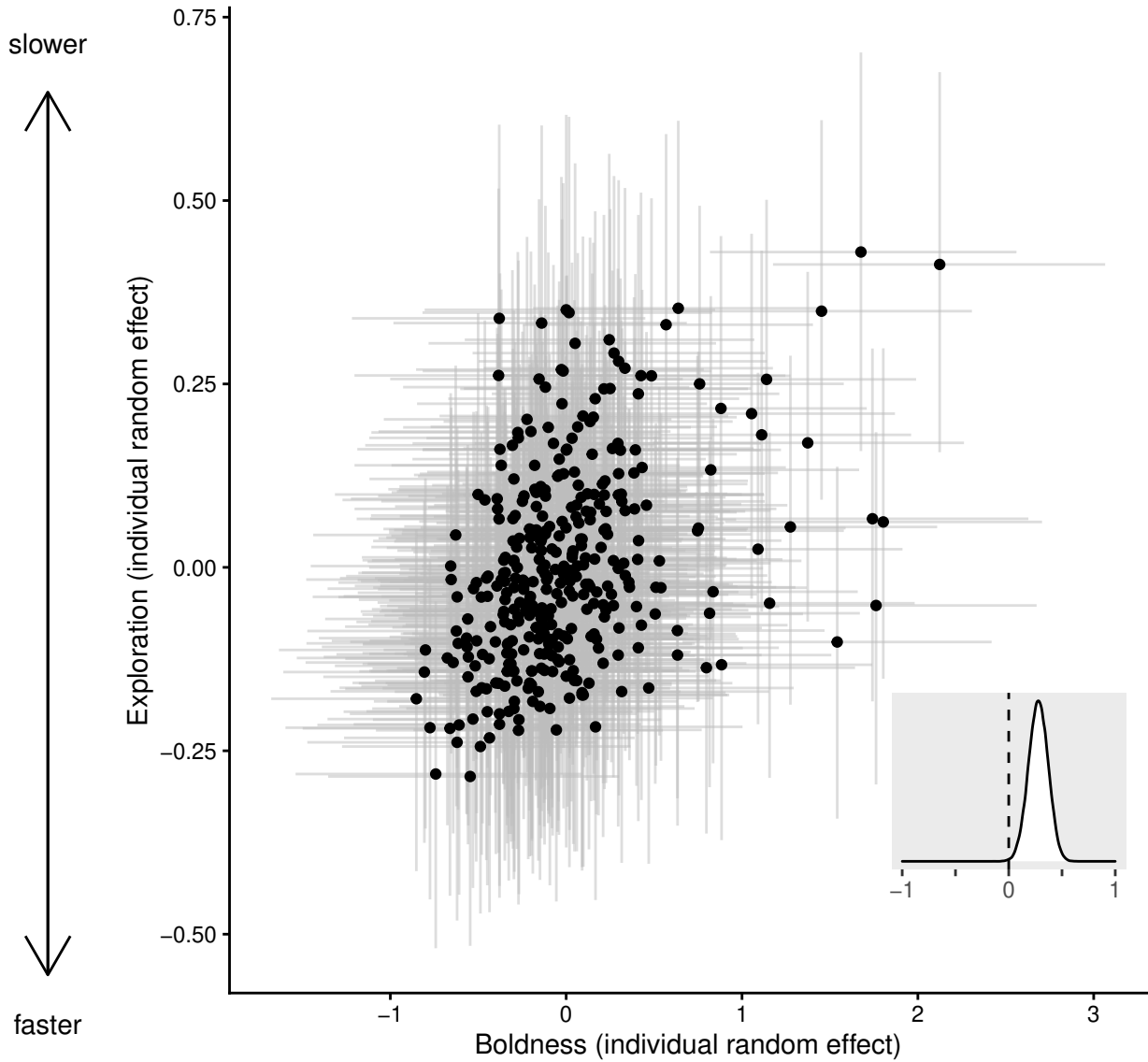
751 **Figure 4.** Correlation between individual-level random effects for boldness and exploration,

752 illustrated by plotting their respective Best Linear Unbiased Predictors and 95% credible intervals.

753 Inset: posterior distribution of the correlation coefficient. BLUPs are plotted, and correlation

754 estimated, on the latent log scale.

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