Morph-dependent effect of nematode infection on host movement in the land snail *Cepaea nemoralis* (Mollusca, Gastropoda)

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Abstract

Host behaviour can be influenced by parasitic risk and infection through a variety of direct and indirect mechanisms. We can expect individuals expressing different phenotypes to also differ in the ways their behaviour is altered by parasites. We used the land snail *Cepaea nemoralis*, a species with various shell colour morphs differing in behaviour and physiology, as a model to study the link between parasite response and individual behaviour variation. We analysed metazoan parasite abundance and its relation to behaviour (movement and food intake) in snails from three shell morphs (from light unbanded to darker five-banded) and from two neighbouring populations from contrasted environments. Snails were parasitized by mites, trematodes and nematodes, from rarest to most frequent. We confirm that terrestrial gastropods can defend against infection by trapping parasitic nematodes in their shell. We show that nematode encapsulation can uncover past infections even when a population is currently nematode-free, possibly due to parasite seasonality. We present new observations suggesting that not only nematodes but also mites might be encapsulated in shells. Vulnerability to parasites was morph-dependent, with darker snails harbouring fewer nematodes. Behaviour (movement and food intake) was dependent on nematode, but not trematode infection. Individuals with higher nematode load ate less, irrespective of morph and population. The morph that was most vulnerable to parasitism (unbanded snails) showed reduced activity in the nematode-infected population compared to the one that was nematode-free at sampling time. As a result, parasite risk may cancel previously described behavioural differences between morphs. We discuss the possible mechanisms explaining morph-dependent responses to parasites, and how parasite risk may be an unseen force shaping *Cepaea nemoralis* morph variation in space and time. We conclude on the possible ecological consequences of the link, mediated by shell colour, between thermal and immune responses.

Keywords: activity; *Brachylaima*; food intake; *Riccardoella*; shell colour polymorphism; snail host-parasite interactions

Introduction

Individuals often differ consistently in their behaviour, including within populations of the same species (Dall, Houston & McNamara, 2004; Réale *et al.*, 2007; Bell, Hankison & Laskowski, 2009; Holtmann, Lagisz & Nakagawa, 2017). A popular idea to explain these differences is that persistent individual differences in behaviour are driven by (and/or co-evolve with) individual variation in "state", generally defined as stable (relative to behaviour) intrinsic phenotypic features (Dall *et al.*, 2004; Sih *et al.*, 2015; Niemelä & Dingemanse, 2018; Cornwell, McCarthy & Biro, 2020). Consistent among-individual variation in behaviour may then persist if the benefits and costs of behaviour vary with state. While this hypothesis has empirical support, the proportion of among-individual behavioural variance explained by any given intrinsic state variable is usually small (Niemelä & Dingemanse, 2018), meaning that explanations based on one or a few state variables are limited. This highlights the need for explanations including both intrinsic state(s) and extrinsic variation in the experienced environment (Niemelä & Dingemanse, 2018).

The boundary between extrinsic and intrinsic "drivers" of behaviours is not always clear-cut, as illustrated by parasites. Indeed, parasites both change the environmental context in which potential hosts express their behaviour, and alter actual hosts' internal state. Focusing on movement-related behaviours as an example (Binning, Shaw & Roche, 2017 for a review), potential hosts may react to the perceived risk of infection by moving to avoid or limit contact with parasites (e.g. Davies & Knowles, 2001). Moreover, costs incurred by infected individuals may lead them to limit or delay movements (e.g. Debeffe *et al.*, 2014; O'Dwyer, Kamiya & Poulin, 2014).

Land snails and slugs host a diverse array of metazoan parasites and parasitoids, including flies, mites, cestodes, trematodes and nematodes (Barker, 2004; Morley & Lewis, 2008; Gracenea & Gállego, 2017; Pieterse, Malan & Ross, 2017; Gérard *et al.*, 2020). Depending on species, parasites use terrestrial gastropods as their only hosts, or as intermediate hosts with birds and mammals often being the definitive hosts (Barker, 2004). Some of these parasites can cause

64 diseases (e.g., strongylosis) in domesticated animals and humans (Meerburg, Singleton &
65 Kijlstra, 2009; Butcher, 2016; Giannelli *et al.*, 2016).

66 Terrestrial gastropods can defend against metazoan parasites in several ways. First, they may
67 alter their behaviour to avoid being infected, by detecting and avoiding parasite-infected micro-
68 habitats (Wynne, Morris & Rae, 2016). If parasites manage to enter snails, they can be
69 neutralized through several mechanisms. The cellular immune response, in which parasites are
70 encapsulated by a large number of amoebocytes, may be associated with a non-cellular
71 response based on the production of cytotoxic molecules (Furuta & Yamaguchi, 2001; Coaglio *et*
72 *al.*, 2018). Snails can also trap nematodes directly in the inner layer of their shell (Rae, 2017).
73 Moreover, as in insects (González-Santoyo & Córdoba-Aguilar, 2012; San-Jose & Roulin, 2018),
74 melanin and the phenoloxidase pathway involved in melanisation may play a key role in snail
75 defences against parasites. For instance, accumulation of melanin around nematodes
76 encapsulated in the haemolymph is documented in the snail *Lissachatina fulica* (Coaglio *et al.*,
77 2018). Phenoloxidase activity has been shown to increase in *L. fulica* and *Helix lucorum* after
78 infection by nematodes or trematodes, respectively (Coaglio *et al.*, 2018; Unlu & Ekici, 2021).
79 While less studied, carotenoids may also contribute to immune responses in invertebrates (Tan
80 *et al.*, 2020). This possible association between immunity and pigment-related pathways could
81 have further ecological relevance in land snails. Indeed, many species exhibit shell colour
82 polymorphism (Williams, 2017), which affects behavioural and physiological thermal responses
83 (Jones, Leith & Rawlings, 1977; Schweizer, Triebskorn & Köhler, 2019; Köhler *et al.*, 2021) or
84 vulnerability to predators (Jones *et al.*, 1977; Rosin *et al.*, 2011; Rosin *et al.*, 2013; Surmacki,
85 Ożarowska-Nowicka & Rosin, 2013).

86 The grove snail *Cepaea nemoralis* has a long history as an iconic model in evolutionary biology
87 (Jones *et al.*, 1977; Ożgo, 2009). Individuals can differ in background shell color (yellow, pink or
88 brown) as well as in the number of dark bands (from zero to five). These differences are
89 genetically determined, and decades of research have aimed to disentangle the roles of thermal
90 selection, predation risk and neutral processes in driving variation in morph frequencies (Cain &
91 Sheppard, 1954; Lamotte, 1959; Jones *et al.*, 1977; Schilthuizen, 2013; Ożgo *et al.*, 2017; Kerstes
92 *et al.*, 2019). These color differences are associated with behavioural and physiological

differences (Kavaliers, 1992; Rosin *et al.*, 2018; Dahirel, Gaudu & Ansart, 2021), as well as other morphological differences (e.g. shell strength, Rosin *et al.*, 2013), leading to potentially adaptive phenotypic syndromes.

If susceptibility and response to parasite infection are shaped by host behaviour and physiology, this means *C. nemoralis* morphs might consistently differ (i) in their vulnerability to parasites and thus the prevalence and strength of infections, and (ii) in the way parasite infection alters host behaviour. In this context, we compared natural infection frequencies by metazoan parasites across two habitats differing in microclimate conditions. We examined whether infections varied with morph and potentially morph-dependent behaviours. We additionally investigated parasite encapsulation across morphs.

Based on the expected link between pigments and immune defence outlined above, we expected snails with more dark bands hosted fewer parasites. Since darker snails are less mobile (Dahirel *et al.*, 2021), that expectation is also in line with some predictions from the pace-of-life syndrome hypothesis, namely that fast active individuals have lower immune responses (Réale *et al.*, 2010; but see Royauté *et al.*, 2018). We also hypothesized that metazoan parasites would have notable negative effects on snail performance (McElroy & de Buron, 2014), leading to slower behaviours and changed food intake in more infected snails (both at the morph level and within morphs). We expected these effects on behaviour to be stronger in morph(s) more vulnerable to parasite infection.

Methods

Snail collection and maintenance

We collected snails in early May 2019 (i.e. at most ≈ 1.5 month after hibernation) in and near the village of Arçais, France. We sampled two previously studied populations, separated by ≈ 2km but differing in abiotic conditions (Dahirel *et al.*, 2021):

- an “open habitat” population (≈ 46°17’50”N, 0°41’30” W), located in a garden with little to no tree and thus exposed to the sun throughout the year. This garden is on relatively calcite-rich substrate (marl, Dupuis *et al.*, 1983; BRGM, 2005).

- a “shaded habitat” population (≈ 46°18’01” N, 0°42’56” W), located in a small humid woodlot which is not directly exposed to the sun during snail activity months, and experiences occasional and partial winter flooding. The substrate is poorer in calcite (Dupuis *et al.*, 1983; BRGM, 2005). Five-banded snails are more frequent in this site, in agreement with hypotheses on shell colour and thermal selection in *C. nemoralis* (Dahirel *et al.*, 2021).

We sampled 90 adult individuals per population, divided equally in three morphs: unbanded, three-banded, and five-banded snails. Adults were recognizable by the presence of a reflected “lip” around the shell opening. As in Dahirel et al. (2021), we only sampled snails with yellow background shells, leaving aside the rarer brown and pink-shelled morphs. Sampling was random with respect to other shell features (e.g. band fusion).

We kept snails under standard conditions (20 ± 1°C, L:D 16:8) in 30 × 45 × 8 cm^3 plastic crates lined with 1-2 cm of soil kept humid and regularly renewed, with a lid made of a fine plastic mesh for aeration. Snails were grouped by morph × population of origin. Food (vegetal flour supplemented with calcium, Hélinove, Saint Paul en Pareds, France) was available *ad libitum* in Petri dishes. Four days before the experiments, we marked snails by writing a unique identifier on the side of their shell (using Posca pens, uni Mitsubishi Pencil, Tokyo, Japan). At the same time, we measured snail size (greater shell diameter) to the nearest 0.1 mm.

Movement behaviour and food intake

Behavioural experiments began two weeks after snail sampling. We tested each snail twice for each trait, to separate repeatable among-individual variation from within-individual variability. To ensure a manageable workload, we split the 180 snails in three groups of 60, each starting the experimental sequence on a different day, with each group containing snails from all band number × population combinations in equal proportions.

We first tested snails for movement behaviour. We stimulated snail activity by placing them in a Petri dish containing a small amount (≈ 1 mm) of water until they were out of their shell. We then placed each snail individually on a clean smooth lab bench, and used the time snails took

147 to move more than 10 cm in a straight line from their starting point as a measure of movement
148 (longer times implying slower individuals). Importantly, we only counted time after snails moved
149 more than 2 cm (i.e. about 1 shell length) from their starting point, to ensure all observed snails
150 were truly active. Some snails stayed inactive and the relevant observations were thus excluded
151 (11 individuals missed one of the two tests, 6 individuals missed both). We stopped observation
152 20 min after placing snails on the lab bench (not after the start of activity).

153 Movement tests were immediately followed by food intake tests. We kept all snails individually
154 in 10 × 7 × 6 cm^3 boxes for 18 hours (including the 8 hours of darkness) and provided them with
155 1.5 g of dry food (a non-limiting quantity, large enough to feed one snail for at least three days,
156 based on preliminary tests both in *C. nemoralis* and the larger *Cornu aspersum*). Afterwards, we
157 recovered, dried (60°C for 60 hours) and weighed the remaining food (to the nearest 0.1 mg),
158 and used the proportion of food consumed out of the initial 1.5 g as our measure of food intake.

159 Snails were then placed back in their original box. The second test for each behaviour was done
160 one week later.

161 **Parasitological research**

162 After snail freezing at -20°C and thawing, we dissected each individual under a binocular
163 microscope. After removing the body from the shell, we looked for and counted metazoan
164 parasites in various locations: space between shell and soft body, lung, heart, kidney, body
165 cavity and digestive gland, as in Gérard et al. (2020). We were able to detect nematodes, larval
166 trematodes and parasitic mites (Acari), covering the main taxa parasitizing helicid land snails (to
167 the exception of parasitoid flies; Barker, 2004). Trematodes were identified to the genus level as
168 in Gérard et al. (2020), and mites using Fain (2004); that level of taxonomic precision could not
169 be reached for nematodes, which remained unidentified. We additionally observed shells and
170 shell fragments under brighter light to detect encapsulated parasites (Rae, 2017). By simplicity,
171 we hereafter use “live” as a shorthand to designate parasites found free within the body or
172 between the body and the shell during dissection, and thus presumably still living before snails
173 were sacrificed, by opposition with “trapped” or “encapsulated” parasites found within shells.

174 **Statistical analyses**

175 We analysed our data in a Bayesian framework, using R (version 4.1.2, R Core Team, 2021) and
176 the *brms* R package (Bürkner, 2017) as frontends for the Stan language (Carpenter *et al.*, 2017).
177 We used the *tidybayes, bayesplot, patchwork* packages for data preparation, model evaluation
178 and plotting, as well as the *tidyverse* suite of packages (Gabry *et al.*, 2019; Kay, 2019; Wickham
179 *et al.*, 2019; Pedersen, 2020). One snail (among the initial 180) died during the experiments and
180 was excluded from statistical analyses, but was used for natural history information (see
181 **Results**).

182 We ran two models, which both included at least effects of population of origin, number of shell
183 bands, as well as population × banding interactions as categorical explanatory variables
184 (hereafter "morph and population effects").

185 We first used a Gaussian linear model to analyse how snail shell diameter (mean centred and
186 scaled to unit 1 SD), varied with morph and population effects.

187 We then ran a multivariate generalized mixed/multilevel model to examine how behaviour and
188 parasite infections varied between morph and populations, and whether these traits were
189 correlated at the individual level. Trematodes and nematodes could not be identified to species,
190 so we used the total numbers of trematodes (i.e. metacercariae of the genus *Brachylaima*) and
191 nematodes per host snail in our analyses (as in e.g. Debeffe *et al.*, 2014). Mite infections were
192 too rare (see **Results**) to be analysed statistically. This multivariate model included four
193 submodels:

194 - live trematode abundance was modelled as a function of morph and population effects,
195 as well as shell size, using a Poisson model. This model included random effects of test
196 group and individual identity. The latter acts as an observation-level random effect and
197 accounts for overdispersion (Harrison, 2014); in addition it allows us to build an
198 individual-level correlation matrix connecting all four traits.
199 - live nematode abundance was modeled as trematode abundance, with one change. As
200 no live nematodes were found in the shaded population, we only fit this submodel to

data from the open population and removed the population fixed effect. Keeping all data instead does not change our conclusions (see associated code, **Data availability**).

- food consumption (mean-centred and scaled to unit 1SD) was analysed using a Gaussian model including the same fixed and random effects as trematode abundance. Although food consumption data are proportions (of total food provided), and so are well-suited to Beta models (Douma & Weedon, 2019), the Gaussian model behaved much better regarding posterior predictive checks (**Data availability**). As individuals were measured twice, the individual random effect here allows separation between within- and among individual variation.
- movement behaviour (latency to move more than 10 cm from the starting point) was inverse-transformed, mean-centred and scaled to unit 1SD, and then analysed using a Gaussian model including the same fixed and random effects as above. We initially attempted to analyse untransformed latencies using a lognormal or Gamma model, but these models again failed to accurately represent the data in posterior predictive checks (see **Data availability**).

In both models we used weakly informative priors (McElreath, 2020): $\mathrm{Normal}(0,1)$ priors for fixed effects, $\mathrm{half-Normal}(0,1)$ priors for standard deviations (random effect and residual), and a $\mathrm{LKJ}(2)$ prior for the correlation matrix in the multivariate model. See **Supporting Information S1** for detailed model descriptions. We ran four chains per model for 4000 iterations each, with the first half of each chain used as warmup. We checked convergence and mixing graphically and using the updated $\hat{R}$ statistic (Vehtari *et al.*, 2021). All parameters had satisfactory effective sample sizes (Vehtari *et al.*, 2021). Posteriors are summarised in text and figures as means [95% Highest Posterior Density Intervals].

We also looked at the proportion of snails with at least one encapsulated nematode in their shell as a function of shell morph and population. Here we did not run statistical models, as we simply wanted to know about past parasite exposure in both populations. We specifically refrained from analysing statistically the prevalence or abundance of encapsulated nematodes per individual, as it is likely linked to cumulative exposure time/age, and we have no

information about snail age and age-dependent or morph-dependent survival in both populations.

Results

Snail size

Snails from the shaded population were on average smaller than their counterparts from the open habitat if they were three-banded (= -0.70 mm [-1.38; -0.04], **Fig. 1**) or five-banded (= -1.37mm [-2.07; -0.71], **Fig. 1**); unbanded snails were however of similar size in both habitats (= -0.39 mm [-1.03; 0.33], **Fig. 1**).

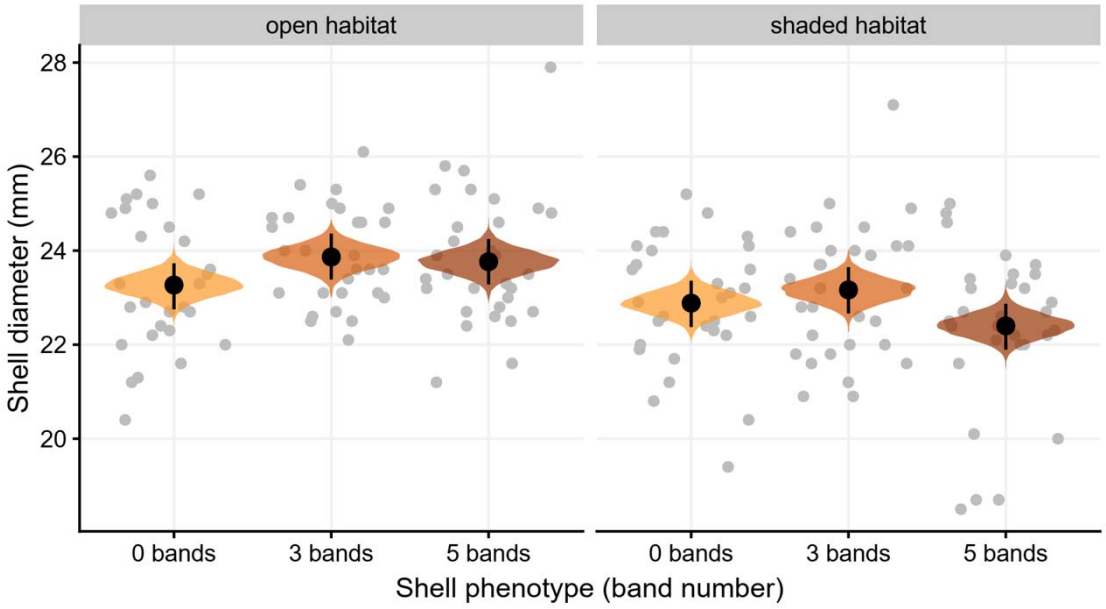
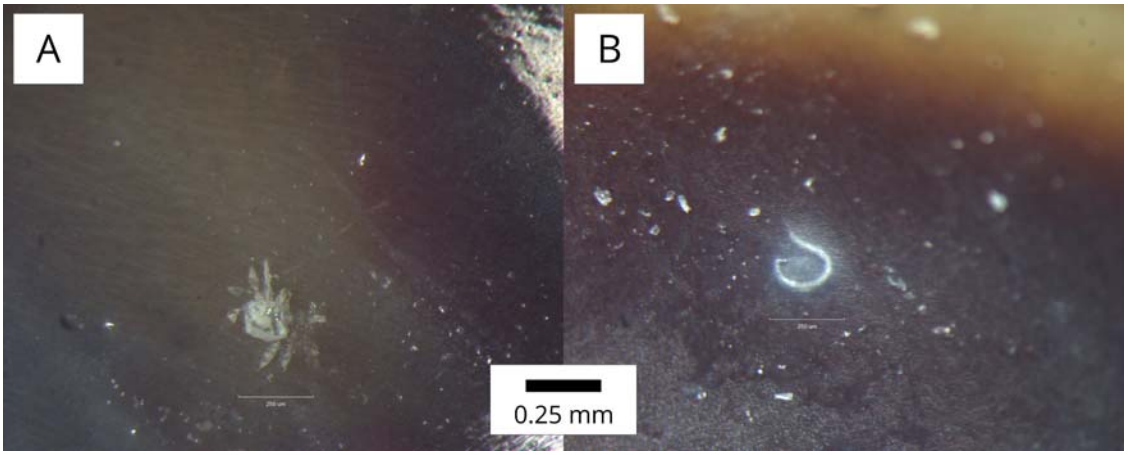


Figure 1. Snail shell diameter as a function of band number and population of origin. Grey dots are observed values, displayed along the posterior distributions of the average sizes (black dots and segments are the means and 95% CI of the posteriors).

Parasitological information

Snails from both populations and all morphs were infected by metazoan parasites (**Figs. 2-3**, see **Supporting Information S2** for detailed descriptive statistics about parasite prevalence and infection intensity). All trematodes found were live *Brachylaima* sp. metacercariae in snails'

245 kidneys; no live sporocysts were recorded and no trematodes of any stage were found trapped
246 in shells. We previously documented *Brachylaima* trematodes infecting another snail species in
247 the same area (Gérard *et al.*, 2020). We detected unidentified nematodes both live in the lung
248 and general cavity of snails and encapsulated in shells. Although no live nematodes were found
249 in individuals from the shaded population (**Fig.3**), a high number of these snails still contained
250 encapsulated nematodes in their shells, independently of morph (**Figs 2, 3**), indicating past
251 exposure to nematodes. Only one snail (unbanded from the shaded habitat) harboured live
252 mites (*N* = 93 mites), identified as *Riccardoella* sp. Three other snails (all five-banded from the
253 shaded habitat) had mites trapped in their shell (*N* = 1, 2 and 15 mites, **Fig. 2**). As mentioned in
254 **Methods**, one snail among 180 died during the experiments; this individual harboured 102 live
255 nematodes, i.e. more than 4 times the number of the most infected surviving snail (**Fig. 3**).

256 

257 **Figure 2**. Examples of encapsulated parasites in *Cepaea nemoralis* shells. (A) mite; (B) nematode. Original
258 images, including other images of encapsulated parasites, are made available with the rest of the data
259 (see **Data availability**).

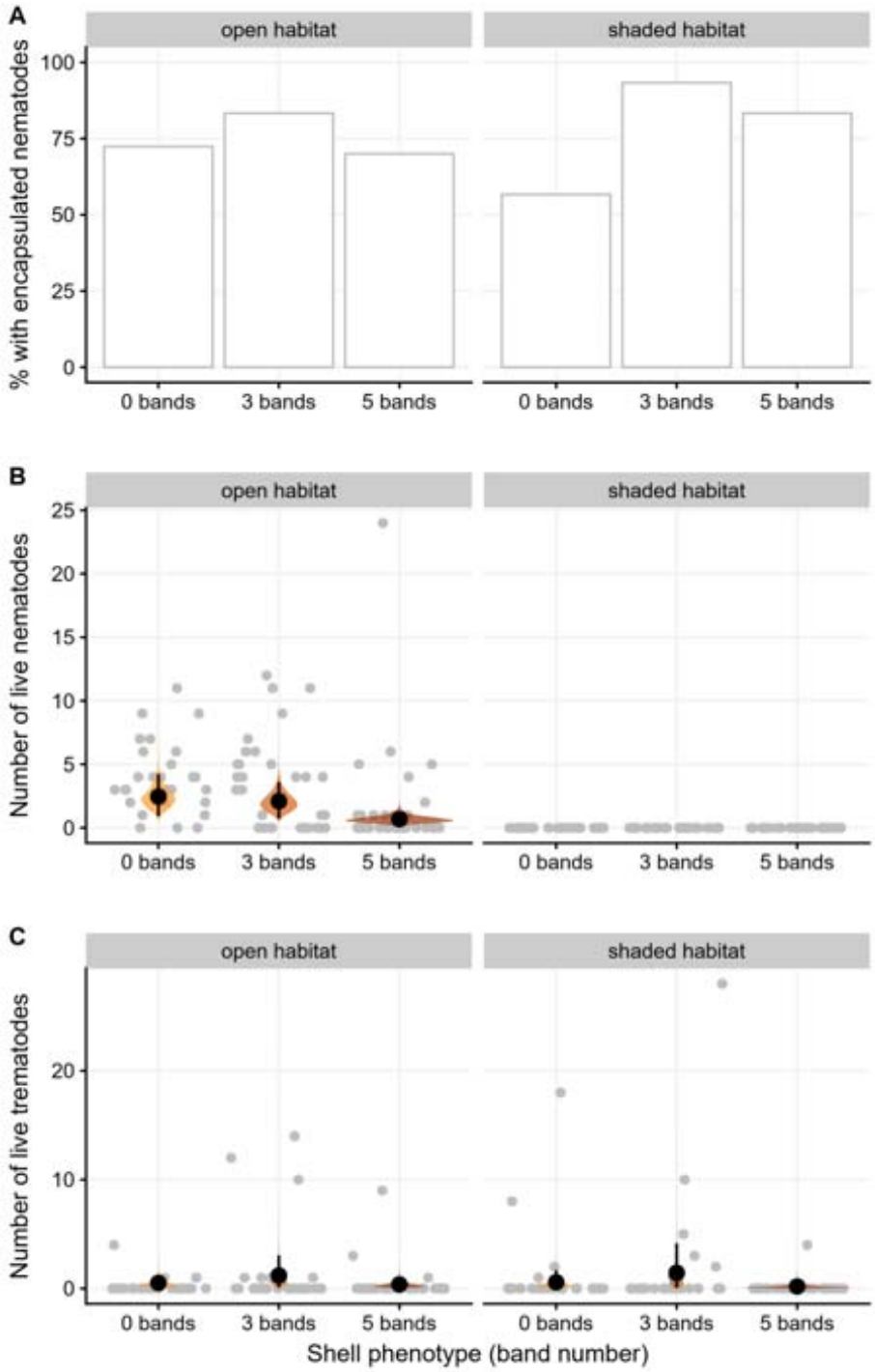


Figure 3. Parasite-related variables as a function of morph and population of origin (*N* = 179, one dead snail was excluded). (A) Proportion of snails having at least one nematode trapped in their shells; (B) Live nematode abundance; (C) Live *Brachylaima* sp. trematode abundance (metacercariae). For (B) and (C): grey dots are observed values, displayed along the posterior distributions of the average abundances

based on the model described **Table 1** (black dots and segments are the means and 95% CI of the posteriors). Posterior predictions are based on fixed effects only, and the effects of shell size are averaged out. See **Supporting Information S3** for pairwise comparisons.

In the open population where live nematodes were found, nematode abundance was morph-dependent: five-banded snails were on average infected by about a third of the nematodes infecting other snails ($N_{[5\text{bands}]}/N_{[0\text{bands}]}$ = 0.29 [0.11; 0.52], $N_{[5\text{bands}]}/N_{[3\text{bands}]}$ = 0.34 [0.12; 0.62]; **Table 1**, **Fig. 3**). Five-banded snails also contained fewer trematodes, but only when compared to three-banded snails and only clearly in the shaded habitat ($N_{[5\text{bands}]}/N_{[3\text{bands}]}$ = 0.19 [0.00; 0.57]; **Fig. 3**, **Supporting Information S3**). Parasite abundance (trematode or nematode) was not linked to shell size (**Table 1**).

Table 1. Summary of posterior model coefficients for the parasite and behaviour multivariate model. Individual-level correlations between traits are presented in **Table 2**. See **Methods** for model details, and **Supporting Information S3** for pairwise comparisons among morph × population groups.

	Nematode abundance*	**Trematode abundance**	**Movement**	**Food intake**
Fixed effects				
Intercept$_{[0\ bands]}$	0.84 [0.09; 1.52]	-0.94 [-2.44; 0.48]	-0.26 [-0.66; 0.16]	-0.22 [-0.61; 0.19]
Intercept$_{[3\ bands]}$	0.67 [-0.08; 1.38]	-0.10 [-1.54; 1.33]	0.16 [-0.24; 0.56]	-0.09 [-0.50; 0.31]
Intercept$_{[5\ bands]}$	-0.47 [-1.31; 0.32]	-1.26 [-2.72; 0.23]	-0.09 [-0.50; 0.31]	-0.04 [-0.45; 0.37]
Population = "shaded"$_{[0\ bands]}$	--	-0.02 [-1.42; 1.39]	0.71 [0.31; 1.08]	0.42 [0.04; 0.83]
Population = "shaded"$_{[3\ bands]}$	--	0.06 [-1.31; 1.34]	-0.23 [-0.61; 0.13]	0.22 [-0.18; 0.63]
Population = "shaded"$_{[5\ bands]}$	--	-0.88 [-2.44; 0.66]	-0.08 [-0.47; 0.32]	0.13 [-0.26; 0.55]
Shell size (standardised)	-0.19 [-0.53; 0.13]	0.06 [-0.57; 0.72]	0.09 [-0.03; 0.21]	0.23 [0.10; 0.35]
Random effects SDs				
Test group SD	0.53 [0.00; 1.23]	1.95 [0.94; 3.19]	0.23 [0.00; 0.70]	0.21 [0.00; 0.67]
Individual-level SD	1.14 [0.85; 1.46]	2.57 [1.99; 3.21]	0.32 [0.04; 0.54]	0.56 [0.43; 0.70]
Residual SD	--	--	0.92 [0.82; 1.02]	0.8 [0.72; 0.88]

* The nematode model is only estimated for the "sun-exposed" population, since no live nematodes were recorded in the "shaded" population

Snail behaviour

Both movement and food intake were repeatable at the individual level (among-individual variance components > 0; **Table 1**), although repeatability was weaker for movement (**Supporting Information S4**).

Movement depended on both population of origin and shell band number (**Table 1**, **Fig. 4**, **Supporting Information S3**). In the shaded population, five-banded snails were on average less mobile than unbanded snails ($\Delta_{[5\text{bands}-0\text{bands}]}$ = -0.61 SDs [-1.00; -0.21]). In snails from the open habitat however, there was no such difference between morphs ($\Delta_{[5\text{bands}-0\text{bands}]}$ = 0.17

SDs [-0.19; 0.59]). More precisely, this was due to unbanded snails being less mobile when coming from the open habitat than when coming from the shaded population ($\Delta_{[\mathrm{shaded-open}]}$ = 0.71 SDs [0.31; 1.08]), while there was no clear between-population difference for five-banded snails ($\Delta_{[\mathrm{shaded-open}]}$ = -0.08 SDs [-0.47; 0.32])(**Table 1**, **Fig. 4**). In any case, there was no detectable link between movement and shell size (**Table 1**).

Larger snails tended to eat more (**Table 1**). Source population had no effect on food intake if snails were three- ($\Delta_{[\mathrm{shaded-open}]}$= 0.03 g [-0.02; 0.08], **Fig. 4**) or five-banded ($\Delta_{[\mathrm{shaded-open}]}$= 0.02 g [-0.03; 0.07], **Fig. 4**), but similarly to movement, unbanded snails coming from the shaded population ate more on average than their counterparts from the open habitat ($\Delta_{[\mathrm{shaded-open}]}$= 0.05 g [0.00; 0.10], **Fig. 4**).

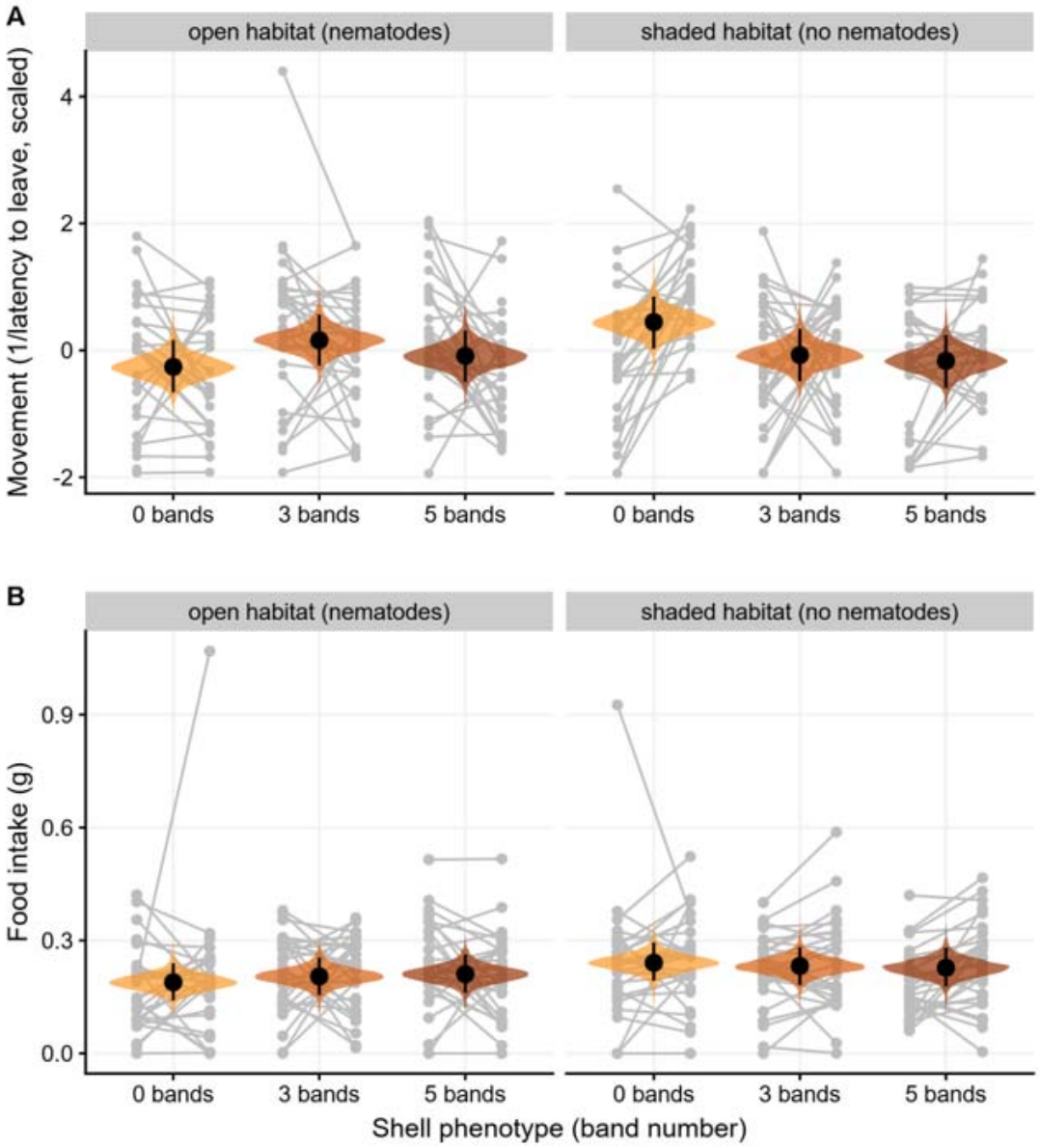


Figure 4. Behaviours as a function of morph and population of origin. (A) Movement metric (1/latency to move beyond 10 cm, centered and scaled to unit 1SD); (B) Food intake. Grey dots are observed values, displayed along the posterior distributions of the average abundances based on the model described in **Table 1** (black dots and segments are the means and 95% CI of the posteriors). Observed values from the same individual are connected by a line. Posterior predictions are based on fixed effects only, and the effects of shell size are averaged out. See **Supporting Information S3** for pairwise comparisons.

Looking at the individual-level correlation matrix between traits (i.e. once fixed effects of shell morph, size and population of origin are accounted for), food intake and nematode abundance are negatively correlated, while all other correlations (including between nematode and trematode abundance) are not markedly different from zero (**Table 2**).

Table 2. Individual-level correlations between parasite abundances and behavioural traits. *N* = 179 individuals for trematode abundance and food intake, *N* = 173 for movement and *N* = 89 for nematode abundance.

	Nematode abundance	**Trematode abundance**	**Movement**
Trematode abundance	0.01 [-0.35; 0.37]	-–	-–
Movement	-0.24 [-0.73; 0.25]	-0.17 [-0.68; 0.28]	-–
Food intake	-0.60 [-0.87; -0.30]	-0.12 [-0.46; 0.19]	0.24 [-0.23; 0.71]

Discussion

To understand how variation in colour morphs is linked to variation in immunity, we studied behaviour (movement and food intake) and natural parasite infection across two populations of the land snail *Cepaea nemoralis*. We found evidence of morph-dependent responses to parasites, as well as links between parasite infection and behaviour. We additionally present what is, to our knowledge, the first report in the literature of land snails encapsulating parasitic arthropods in their shell.

Snails of the darker morphs were smaller in the shaded population (**Fig. 1**). While the two habitats differ along various abiotic dimensions (calcium availability in substrate, microclimate…, see **Methods**), their documented effect on adult shell size are highly species- and study-dependent, and there is no consistent evidence for or against their role in driving our observed body size differences (Goodfriend, 1986). Alternately, differences in parasite infection between the two habitats might also influence body size, as infection before maturity may stunt growth (Gérard & Théron, 1997), and high parasite risk may drive resource allocation away from growth and towards immune defence, if there is a trade-off between the two (e.g. Rantala &

332 Roff, 2005). Here in any case, our models conditioned on size, meaning that any effect (or
333 absence of effect) of morph/population we discuss below should be assumed to be "size being
334 equal". In addition, we did not find any effect of size itself on parasite abundance or movement
335 (**Table 1**), meaning that the effects of morph/population we found are likely not mediated by
336 size differences.

337 Although only one snail was infected by live *Riccardoella* mites, we found encapsulated mites in
338 three others (**Fig. 2**). Many land snails are able to defend against infection by parasitic
339 nematodes by trapping them in their shell (Rae, 2017), including *Cepaea nemoralis* (this study,
340 Williams & Rae, 2016; Rae, 2017). Rae (2017) affirmed that encapsulation in the shell is
341 specifically a response against nematode infection, drawing on the absence of other parasites
342 across their large sample of shells; our results might contradict this affirmation. *Riccardoella*
343 infection is highly variable across populations or species, with reported prevalences of 0% or
344 near 0% being frequently observed (this study; Russo & Madec, 2013; Baur & Baur 2005; Segade
345 *et al.* 2013). Combined with the fact that encapsulation in the shell clearly has a success
346 probability <1 (in our data, ≈ 22% of snails infected by nematodes at the time of the experiment
347 showed no traces of nematode encapsulations; **Data availability**), this may explain why
348 encapsulated mites were not previously found despite extensive surveys. We however need
349 further studies to determine with certainty whether shell encapsulation is a more general
350 response to metazoan parasites or if it is a nematode-specific response, as posited by Rae
351 (2017), but occasionally and anecdotally catches other parasites.

352 We found no live nematodes in any snail from the shaded population (**Fig. 3**; note that our
353 methods were not designed to detect species such as *Alloionema appendiculatum*, which are
354 embedded in foot tissue; Morand, Wilson & Glen, 2004). Thanks to nematodes trapped in shells
355 (**Fig. 3**), which integrate infections over time, we know that this was not because parasitic
356 nematodes are absent from the shaded population; they were simply not infecting snails at the
357 time of sampling. The "shaded" woodlot habitat is sheltered from the sun, more humid and
358 likely colder than the open one. Although it can be highly variable in time and space (Sweeny *et*
359 *al.*, 2021), infections are often dependent on abiotic parameters, including temperature and
360 humidity, leading to seasonality (Altizer *et al.*, 2006). Differences in nematode infection

361 between the habitats may then be explained, at least partly, by differences in microclimate. In
362 particular, conditions favourable to nematode activity, development or infection may be
363 reached only later in the year in the shaded habitat. For instance, in a field trial, *Deroceras*
364 *reticulatum* slugs were more negatively impacted by *Phasmarhabditis hermaphrodita* infection
365 when conditions were artificially warmed during winter (El-Danasoury & Iglesias-Piñeiro, 2017),
366 indicating that warmer conditions may favour nematode activity. In any case, these results
367 mean that any between-population differences in behaviour in the present study need to be
368 interpreted not only in relation with microhabitat and microclimate differences, but also in
369 terms of average infection levels.

370 In the open population where active nematodes were actually found, morph differences in
371 infection seem to validate our initial hypothesis of increased immune defence in darker snails
372 (**Fig. 3**). By contrast, no significant morph difference in immune defence was found in the closely
373 related *Cepaea hortensis* (Scheil *et al.*, 2013, 2014), but their endpoints were different
374 (phenoloxidase activity in 2013 and mortality in 2014). In addition, our behavioural results
375 (discussed below) are also consistent with the hypothesis of morph-dependent immunity. These
376 results are interesting in the light of recent evidence that melanins may not be the pigments
377 forming the dark bands in *C. nemoralis* (Affenzeller *et al.*, 2020). Indeed, our initial hypothesis
378 when designing this experiment (in 2019) was based on the oft-seen association between
379 immunity and melanin (San-Jose & Roulin, 2018), and on the role of melanin in nematode
380 defence in e.g. *Lissachatina fulica* (Coaglio *et al.*, 2018), although other pigments may also have
381 links to immunity (Tan *et al.*, 2020). While it is still likely that melanin-associated pathways play
382 a role in immune defence in *Cepaea* (Scheil *et al.*, 2014), the actual mechanism is probably not
383 as simple as "banded snails constitutively produce more melanin, and thus are better
384 protected". More studies are needed to understand the mechanism linking shell morph to
385 nematode resistance.

386 By contrast, we found no evidence that morph or population had any influence on the
387 abundance of trematodes (**Fig. 3**). These results are consistent with another study analyzing
388 both *C. nemoralis* and *C. hortensis* (Żbikowska *et al.*, 2020). We must acknowledge however that
389 snail infection by trematodes is highly seasonal: infections by *Brachylaima* seem to be at their

390 highest in fall (Gracenea & Gállego, 2017), while we sampled during spring. Longitudinal studies,
391 possibly combined with experimental infections or cures, may help confirm whether morphs
392 respond differently or not to trematode risk.

393 Nematode and trematode numbers were uncorrelated (**Table 2**). Terrestrial gastropods are
394 commonly infected by both nematodes and trematodes at the same time (e.g. Segade *et al.*,
395 2013; Antzée-Hyllseth *et al.*, 2020). Our results extend these previous studies, and suggest that
396 despite sharing a host, nematode and trematode niches are sufficiently distinct within snails to
397 limit spatial and trophic competition. We reiterate however that studies more expansive in
398 space and time, including seasons and/or populations with higher prevalence and abundance of
399 trematodes and nematodes, are needed to confirm this. Combined with finer-grained studies
400 (e.g. at the parasite species level; Morand *et al.*, 2004), this would allow us to understand better
401 how parasites with similar or different niches within snails interact.

402 We found that movement behaviour in *C. nemoralis* was morph-dependent, but that this
403 dependency varied between populations: five-banded morphs were less mobile, but contrary to
404 Dahirel et al. (2021), this was only the case in the shaded population (**Fig. 4**). In the open
405 population, snail morphs are mobile at similarly low levels: five-banded snails do not change
406 behaviour between the two populations, while unbanded snails go down to five-banded snail
407 levels of mobility (**Fig. 4**). One of the main differences between the present study and Dahirel et
408 al. (2021) is the collection date. While here snails were collected several weeks after the onset
409 of spring activity and kept active throughout, in Dahirel et al. (2021) they were collected in late
410 fall and stored at cold temperatures inducing dormancy for months before being tested. This
411 difference may be especially relevant: winter dormancy leads to sharp decreases in ciliate and
412 nematode infection in farmed *Cornu aspersum* snails (Segade *et al.*, 2013; but see Morelli *et al.*,
413 2020 for a species-specific possible counter-example), or mite infection in *Arianta arbustorum*
414 (Haeussler *et al.*, 2012). Aestivation also slows down parasite development in two smaller
415 species of land snails (*Theba pisana* and *Trochoidea seetzenii*), and reduces parasite numbers in
416 the latter (Solomon, Paperna & Matkovics, 1996). We can thus reconcile the behavioral results
417 from Dahirel et al. (2021) from those of the present study if we assume that in the former
418 experiment, hibernation reduced parasite load across all morphs and populations, and that in

419 the present study, lower temperatures in the shaded habitat led to longer hibernation. We can
420 then interpret population differences in the present study in the light of their different infection
421 patterns. Five-banded snails' behaviour is almost unchanged between the two populations,
422 while unbanded snails from the "infected" population are slower than in the nematode-free
423 population (**Fig. 4**). This is again consistent with the idea that unbanded snails are more
424 vulnerable to parasites than banded ones.

425 Food intake was influenced by nematode infection: highly parasitized snails ate less (**Table 2**).
426 Feeding reduction in nematode-infected individuals has been documented in other terrestrial
427 mollusc species (Antzée-Hyllseth *et al.*, 2020; Saeedizadeh & Niasti, 2020) and is an indication
428 that parasite infection is indeed costly for snails even if they are non-lethal, at least in adults
429 (Wilson *et al.*, 2000; Williams & Rae, 2016; Grannell, Cutler & Rae, 2021). By contrast,
430 trematode infection had no effect on behaviour, including feeding behaviour (**Table 2**). We
431 however only found snails infected by *Brachylaima* metacercariae (in the kidney). At the
432 sporocyst stage, *Brachylaima* parasitizes the digestive gland (e.g. Gérard *et al.*, 2020), which
433 would likely have a stronger effect on snail behaviour. Indeed, sporocysts can cause high
434 damage, direct and indirect, to that key organ for digestion and for energy storage (Segade *et*
435 *al.*, 2011; Żbikowska *et al.*, 2020).

436 The fact that snail response to parasites seems to be morph-dependent for nematodes, but not
437 for trematodes, has interesting implications for host-parasite and snail-predator interactions in
438 a changing world. Trematodes infecting helicid land snails are trophically transmitted to
439 vertebrate definitive hosts including rodents and birds (Gérard *et al.*, 2020; Sitko & Heneberg,
440 2020). Visual selection by thrushes (*Turdus philomelos*) has been advanced as one of the key
441 explanations for the maintenance of *Cepaea* shell polymorphism (Jones *et al.*, 1977), and
442 predation by rodents may also be morph dependent (Rosin *et al.*, 2011; Rosin *et al.*, 2013). If
443 confirmed, our results suggest that morph-dependent predation confers no additional
444 trematode-avoiding advantage. In addition, snail morphs differ in their thermal responses:
445 lighter-coloured morphs are better adapted to heat, and increase in frequency with global
446 warming or urbanization (Jones *et al.*, 1977; Silvertown *et al.*, 2011; Ożgo *et al.*, 2017; Kerstes *et*
447 *al.*, 2019). If lighter morphs are more vulnerable to infection by nematodes, then we need to

448 understand how snail immunity will interact with thermal tolerances to jointly shape snail and
449 nematode dynamics (El-Danasoury & Iglesias-Piñeiro, 2017; Aleuy & Kutz, 2020), especially
450 given potential risks to public and veterinary health (Meerburg *et al.*, 2009; Giannelli *et al.*,
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456 **Data availability**

457 Data and R scripts to reproduce all analyses presented in this manuscript are available on Github
458 (https://github.com/mdahirel/cepaea-parasite-behaviour-2019) and archived in Zenodo (DOI:
459 https://doi.org/10.5281/zenodo.6327265). Representative images of parasites trapped in shells
460 are also archived in Zenodo (DOI: https://doi.org/10.5281/zenodo.6326305).

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464 **References**

465 Affenzeller, S., Wolkenstein, K., Frauendorf, H. & Jackson, D.J. (2020). Challenging the concept
466 that eumelanin is the polymorphic brown banded pigment in *Cepaea nemoralis*. *Sci. Rep.* **10**,
467 2442.

468 Aleuy, O.A. & Kutz, S. (2020). Adaptations, life-history traits and ecological mechanisms of
469 parasites to survive extremes and environmental unpredictability in the face of climate change.
470 *Int. J. Parasitol. Parasites Wildl.* **12**, 308–317.

471 Altizer, S., Dobson, A., Hosseini, P., Hudson, P., Pascual, M. & Rohani, P. (2006). Seasonality and
472 the dynamics of infectious diseases. *Ecol. Lett.* **9**, 467–484.

473 Antzée-Hyllseth, H., Trandem, N., Torp, T. & Haukeland, S. (2020). Prevalence and parasite load
474 of nematodes and trematodes in an invasive slug and its susceptibility to a slug parasitic
475 nematode compared to native gastropods. *J. Invertebr. Pathol.* **173**, 107372.

476 Barker, G.M. (Ed.). (2004). *Natural enemies of terrestrial molluscs*. Wallingford, UK: CABI.

477 Baur, A. & Baur, B. (2005). Interpopulation variation in the prevalence and intensity of parasitic
478 mite infection in the land snail *Arianta arbustorum*. *Invertebr. Biol.* **124**, 194–201.

479 Bell, A.M., Hankison, S.J. & Laskowski, K.L. (2009). The repeatability of behaviour: a meta-
480 analysis. *Anim. Behav.* **77**, 771–783.

481 Binning, S.A., Shaw, A.K. & Roche, D.G. (2017). Parasites and host performance: incorporating
482 infection into our understanding of animal movement. *Integr. Comp. Biol.* **57**, 267–280.

483 BRGM. (2005). Geological map at 1:50 000 (BD-CHARM-50), department: Deux-Sèvres (79,
484 France).

485 Bürkner, P.-C. (2017). brms: an R package for Bayesian multilevel models using Stan. *J. Stat.*
486 *Softw.* **80**, 1–28.

487 Butcher, A.R. (2016). Children, snails and worms: the *Brachylaima cribbi* story. *Microbiol. Aust.*
488 **37**, 30–33.

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

490 Carpenter, B., Gelman, A., Hoffman, M.D., Lee, D., Goodrich, B., Betancourt, M., Brubaker, M.,
491 Guo, J., Li, P. & Riddell, A. (2017). Stan: a probabilistic programming language. *J. Stat. Softw.* **76**,
492 1–32.

493 Coaglio, A.L., Ferreira, M.A.N.D., dos Santos Lima, W. & de Jesus Pereira, C.A. (2018).
494 Identification of a phenoloxidase- and melanin-dependent defence mechanism in *Achatina*
495 *fulica* infected with *Angiostrongylus vasorum*. *Parasites Vectors* **11**, 113.

496 Cornwell, T.O., McCarthy, I.D. & Biro, P.A. (2020). Integration of physiology, behaviour and life
497 history traits: personality and pace of life in a marine gastropod. *Anim. Behav.* **163**, 155–162.

498 Dahirel, M., Gaudu, V. & Ansart, A. (2021). Boldness and exploration vary between shell morphs
499 but not environmental contexts in the snail *Cepaea nemoralis*. *Ethology* 13.

500 Dall, S.R.X., Houston, A.I. & McNamara, J.M. (2004). The behavioural ecology of personality:
501 consistent individual differences from an adaptive perspective. *Ecol. Lett.* **7**, 734–739.

502 Davies, M.S. & Knowles, A.J. (2001). Effects of trematode parasitism on the behaviour and
503 ecology of a common marine snail (*Littorina littorea* (L.)). *J. Exp. Mar. Biol. Ecol.* **260**, 155–167.

504 Debeffe, L., Morellet, N., Verheyden-Tixier, H., Hoste, H., Gaillard, J.-M., Cargnelutti, B., Picot,
505 D., Sevila, J. & Hewison, A.J.M. (2014). Parasite abundance contributes to condition-dependent
506 dispersal in a wild population of large herbivore. *Oikos* **123**, 1121–1125.

507 Douma, J.C. & Weedon, J.T. (2019). Analysing continuous proportions in ecology and evolution:
508 a practical introduction to beta and Dirichlet regression. *Methods Ecol. Evol.* **10**, 1412–1430.

509 Dupuis, J., Cariou, E., Coirier, B. & Ducloux, J. (1983). *Notice explicative, Carte géologique de la*
510 *France à 1/50 000, feuille Niort (610)*. Orléans, France: BRGM.

511 El-Danasoury, H. & Iglesias-Piñeiro, J. (2017). Performance of the slug parasitic nematode
512 *Phasmarhabditis hermaphrodita* under predicted conditions of winter warming. *J. Pestic.*
513 *Science* **42**, 62–66.

514 Fain, A. (2004). Mites (Acari) parasitic and predaceous on terrestrial gastropods. In *Natural*
515 *enemies of terrestrial molluscs*: 505–524. Barker, G.M. (Ed.). Wallingford, UK: CABI.

516 Furuta, E. & Yamaguchi, K. (2001). Haemolymph: blood cell morphology and function. In *The*
517 *biology of terrestrial molluscs*: 289–306. Barker, G.M. (Ed.). Wallingford, UK: CABI.

518 Gabry, J., Simpson, D., Vehtari, A., Betancourt, M. & Gelman, A. (2019). Visualization in Bayesian
519 workflow. *J. Royal Stat. Soc. A* **182**, 389–402.

520 Gérard, C., Ansart, A., Decanter, N., Martin, M.-C. & Dahirel, M. (2020). *Brachylaima* spp.
521 (Trematoda) parasitizing *Cornu aspersum* (Gastropoda) in France with potential risk of human
522 consumption. *Parasite* **27**, 15.

523 Gérard, C. & Théron, A. (1997). Age/size- and time-specific effects of *Schistosoma mansoni* on
524 energy allocation patterns of its snail host *Biomphalaria glabrata*. *Oecologia* **112**, 447–452.

525 Giannelli, A., Cantacessi, C., Colella, V., Dantas-Torres, F. & Otranto, D. (2016). Gastropod-borne
526 helminths: a look at the snail–parasite interplay. *Trends Parasitol.* **32**, 255–264.

527 González-Santoyo, I. & Córdoba-Aguilar, A. (2012). Phenoloxidase: a key component of the
528 insect immune system. *Entomol. Exp. Appl.* **142**, 1–16.

529 Goodfriend, G.A. (1986). Variation in land-snail shell form and size and its causes: a review. *Syst.*
530 *Zool.* **35**, 204–223.

531 Gracenea, M. & Gállego, L. (2017). Brachylaimiasis: *Brachylaima* spp. (Digenea: Brachylaimidae)
532 metacercariae parasitizing the edible snail *Cornu aspersum* (Helicidae) in Spanish public
533 marketplaces and health-associated risk factors. *J. Parasitol.* **103**, 440–450.

534 Grannell, A., Cutler, J. & Rae, R. (2021). Size-susceptibility of *Cornu aspersum* exposed to the
535 malacopathogenic nematodes *Phasmarhabditis hermaphrodita* and *P. californica*. *Biocontrol Sci.*
536 *Technol.* **31**, 1149–1160.

537 Haeussler, E.M., Pizá, J., Schmera, D. & Baur, B. (2012). Intensity of parasitic mite infection
538 decreases with hibernation duration of the host snail. *Parasitology* **139**, 1038–1044.

539 Harrison, X.A. (2014). Using observation-level random effects to model overdispersion in count
540 data in ecology and evolution. *PeerJ* **2**, e616.

541 Holtmann, B., Lagisz, M. & Nakagawa, S. (2017). Metabolic rates, and not hormone levels, are a
542 likely mediator of between-individual differences in behaviour: a meta-analysis. *Funct. Ecol.* **31**,
543 685–696.

544 Jones, J.S., Leith, B.H. & Rawlings, P. (1977). Polymorphism in *Cepaea*: a problem with too many
545 solutions? *Annu. Rev. Ecol. Evol. Syst.* **8**, 109–143.

546 Kavaliers, M. (1992). Opioid systems, behavioral thermoregulation and shell polymorphism in
547 the land snail, *Cepaea nemoralis*. *J. Comp. Physiol. B* **162**, 172–178.

548 Kay, M. (2019). *tidybayes: tidy data and geoms for Bayesian models*.
549 https://doi.org/10.5281/zenodo.1308151

550 Kerstes, N.A.G., Breeschoten, T., Kalkman, V.J. & Schilthuizen, M. (2019). Snail shell colour
551 evolution in urban heat islands detected via citizen science. *Commun. Biol.* **2**, 264.

552 Köhler, H.-R., Capowiez, Y., Mazzia, C., Eckstein, H., Kaczmarek, N., Bilton, M.C., Burmester,
553 J.K.Y., Capowiez, L., Chueca, L.J., Favilli, L., Gomila, J.F., Manganelli, G., Mazzuca, S.,
554 Moreno-Rueda, G., Peschke, K., Piro, A., Cardona, J.Q., Sawallich, L., Staikou, A.E., Thomassen,
555 H.A. & Triebskorn, R. (2021). Experimental simulation of environmental warming selects against
556 pigmented morphs of land snails. *Ecol. Evol.* **11**, 1111–1130.

557 Lamotte, M. (1959). Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring*
558 *Harb. Symp. Quant. Biol.* **24**, 65–86.

559 McElreath, R. (2020). *Statistical rethinking: a Bayesian course with examples in R and Stan*. 2nd
560 edition. Boca Raton, USA: Chapman and Hall/CRC.

561 McElroy, E.J. & de Buron, I. (2014). Host performance as a target of manipulation by parasites: a
562 meta-analysis. *J. Parasitol.* **100**, 399–410.

563 Meerburg, B.G., Singleton, G.R. & Kijlstra, A. (2009). Rodent-borne diseases and their risks for
564 public health. *Crit. Rev. Microbiol.* **35**, 221–270.

565 Morand, S., Wilson, M.J. & Glen, D.M. (2004). Nematodes (Nematoda) parasitic in terrestrial
566 molluscs. In *Natural enemies of terrestrial molluscs*: 525–557. Barker, G.M. (Ed.). Wallingford,
567 UK: CABI.

568 Morelli, S., Traversa, D., Colombo, M., Raue, K., Strube, C., Pollmeier, M. & Di Cesare, A. (2020).
569 The effect of the hibernation on the larval development of *Troglostrongylus brevior* in the land
570 snail *Cornu aspersum*. *Vet. Parasitol.* **282**, 109123.

571 Morley, N.J. & Lewis, J.W. (2008). The influence of climatic conditions on long-term changes in
572 the helminth fauna of terrestrial molluscs and the implications for parasite transmission in
573 southern England. *J. Helminthol.* **82**, 325–335.

574 Niemelä, P.T. & Dingemanse, N.J. (2018). Meta-analysis reveals weak associations between
575 intrinsic state and personality. *Proc. R. Soc. B: Biol. Sci.* **285**, 20172823.

576 O'Dwyer, K., Kamiya, T. & Poulin, R. (2014). Altered microhabitat use and movement of littorinid
577 gastropods: the effects of parasites. *Mar. Biol.* **161**, 437–445.

578 Ożgo, M. (2009). Current problems in the research of *Cepaea* polymorphism. *Folia Malacol.* **16**,
579 55–60.

580 Ożgo, M., Liew, T.-S., Webster, N.B. & Schilthuizen, M. (2017). Inferring microevolution from
581 museum collections and resampling: lessons learned from *Cepaea*. *PeerJ* **5**, e3938.

582 Pedersen, T.L. (2020). *patchwork: the composer of plots*. https://CRAN.R-
583 project.org/package=patchwork

584 Pieterse, A., Malan, A.P. & Ross, J.L. (2017). Nematodes that associate with terrestrial molluscs
585 as definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its
586 development as a biological molluscicide. *J. Helminthol.* **91**, 517–527.

587 R Core Team. (2021). *R: a language and environment for statistical computing*. Vienna, Austria:
588 R Foundation for Statistical Computing.

589 Rae, R. (2017). The gastropod shell has been co-opted to kill parasitic nematodes. *Sci. Rep.* **7**,
590 4745.

591 Rantala, M.J. & Roff, D.A. (2005). An analysis of trade-offs in immune function, body size and
592 development time in the Mediterranean Field Cricket, *Gryllus bimaculatus*. *Funct. Ecol.* **19**, 323–
593 330.

594 Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V. & Montiglio, P.-O. (2010).
595 Personality and the emergence of the pace-of-life syndrome concept at the population level.
596 *Phil. Trans. R. Soc. B* **365**, 4051–4063.

597 Réale, D., Reader, S.M., Sol, D., McDougall, P.T. & Dingemanse, N.J. (2007). Integrating animal
598 temperament within ecology and evolution. *Biol. Rev.* **82**, 291–318.

599 Rosin, Z.M., Kobak, J., Lesicki, A. & Tryjanowski, P. (2013). Differential shell strength of *Cepaea*
600 *nemoralis* colour morphs—implications for their anti-predator defence. *Naturwissenschaften*
601 **100**, 843–851.

602 Rosin, Z.M., Kwieciński, Z., Lesicki, A., Skórka, P., Kobak, J., Szymańska, A., Osiejuk, T.S., Kałuski,
603 T., Jaskulska, M. & Tryjanowski, P. (2018). Shell colour, temperature, (micro)habitat structure
604 and predator pressure affect the behaviour of *Cepaea nemoralis*. *Sci. Nat.* **105**, 35.

605 Rosin, Z.M., Olborska, P., Surmacki, A. & Tryjanowski, P. (2011). Differences in predatory
606 pressure on terrestrial snails by birds and mammals. *J. Biosci.* **36**, 691–699.

607 Royauté, R., Berdal, M.A., Garrison, C.R. & Dochtermann, N.A. (2018). Paceless life? A meta-
608 analysis of the pace-of-life syndrome hypothesis. *Behav. Ecol. Sociobiol.* **72**, 64.

609 Russo, J. & Madec, L. (2013). Linking immune patterns and life history shows two distinct
610 defense strategies in land snails (Gastropoda, Pulmonata). *Physiol. Biochem. Zool.* **86**, 193–204.

611 Saeedizadeh, A. & Niasti, F. (2020). Response of grey slug to entomopathogenic nematodes.
612 *Bragantia* **79**, 447–456.

613 San-Jose, L.M. & Roulin, A. (2018). Toward understanding the repeated occurrence of
614 associations between melanin-based coloration and multiple phenotypes. *Am. Nat.* **192**, 111–
615 130.

616 Scheil, A.E., Hilsmann, S., Triebskorn, R. & Köhler, H.-R. (2013). Shell colour polymorphism,
617 injuries and immune defense in three helicid snail species, *Cepaea hortensis*, *Theba pisana* and
618 *Cornu aspersum maximum*. *Results Immunol.* **3**, 73–78.

619 Scheil, A.E., Hilsmann, S., Triebskorn, R. & Köhler, H.-R. (2014). Shell colouration and parasite
620 tolerance in two helicoid snail species. *J. Invertebr. Pathol.* **117**, 1–8.

621 Schilthuizen, M. (2013). Rapid, habitat-related evolution of land snail colour morphs on
622 reclaimed land. *Heredity* **110**, 247–252.

623 Schweizer, M., Triebskorn, R. & Köhler, H.-R. (2019). Snails in the sun: strategies of terrestrial
624 gastropods to cope with hot and dry conditions. *Ecol. Evol.* **9**, 12940–12960.

625 Segade, P., Crespo, C., García, N., García-Estévez, J.M., Arias, C. & Iglesias, R. (2011).
626 *Brachylaima aspersae* n. sp. (Digenea: Brachylaimidae) infecting farmed snails in NW Spain:
627 Morphology, life cycle, pathology, and implications for heliciculture. *Vet. Parasitol.* **175**, 273–
628 286.

629 Segade, P., García-Estévez, J., Arias, C. & Iglesias, R. (2013). Parasitic infections in mixed system-
630 based heliciculture farms: dynamics and key epidemiological factors. *Parasitology* **140**, 482–497.

631 Sih, A., Mathot, K.J., Moirón, M., Montiglio, P.-O., Wolf, M. & Dingemanse, N.J. (2015). Animal
632 personality and state–behaviour feedbacks: a review and guide for empiricists. *Trends Ecol. Evol.*
633 **30**, 50–60.

634 Silvertown, J., Cook, L., Cameron, R., Dodd, M., McConway, K., Worthington, J., Skelton, P.,
635 Anton, C., Bossdorf, O., Baur, B., Schilthuizen, M., Fontaine, B., Sattmann, H., Bertorelle, G.,
636 Correia, M., Oliveira, C., Pokryszko, B., Ożgo, M., Stalažs, A., Gill, E., Rammul, Ü., Sólymos, P.,
637 Féher, Z. & Juan, X. (2011). Citizen science reveals unexpected continental-scale evolutionary
638 change in a model organism. *PLOS ONE* **6**, e18927.

639 Sitko, J. & Heneberg, P. (2020). Emerging helminthiases of song thrush (*Turdus philomelos*) in
640 Central Europe. *Parasitol Res.* **119**, 4123–4134.

641 Solomon, A., Paperna, I. & Matkovics, A. (1996). The influence of aestivation in land snails on
642 the larval development of *Muellerius* cf. *capillaris* (Metastrongyloidea: Protostrongylidae). *Int. J.*
643 *Parasitol.* **26**, 363–367.

644 Surmacki, A., Ożarowska-Nowicka, A. & Rosin, Z.M. (2013). Color polymorphism in a land snail
645 *Cepaea nemoralis* (Pulmonata: Helicidae) as viewed by potential avian predators.
646 *Naturwissenschaften* **100**, 533–540.

647 Sweeny, A.R., Albery, G.F., Venkatesan, S., Fenton, A. & Pedersen, A.B. (2021). Spatiotemporal
648 variation in drivers of parasitism in a wild wood mouse population. *Funct. Ecol.* **35**, 1277–1287.

649 Tan, K., Zhang, H., Lim, L.-S., Ma, H., Li, S. & Zheng, H. (2020). Roles of carotenoids in
650 invertebrate immunology. *Front. Immunol.* **10**.

651 Unlu, A.H. & Ekici, A. (2021). Phenoloxidase is involved in the immune reaction of *Helix lucorum*
652 to parasitic infestation by dicrocoeliid trematode. *Ann. Agric. Environ. Med.* **28**, 426–429.

653 Vehtari, A., Gelman, A., Simpson, D., Carpenter, B. & Bürkner, P.-C. (2021). Rank-normalization,
654 folding, and localization: an improved $\hat{R}$ for assessing convergence of MCMC (with discussion).
655 *Bayesian Anal.* **16**, 667–718.

656 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G.,
657 Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J.,
658 Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K. & Yutani, H.
659 (2019). Welcome to the Tidyverse. *J. Open Source Softw.* **4**, 1686.

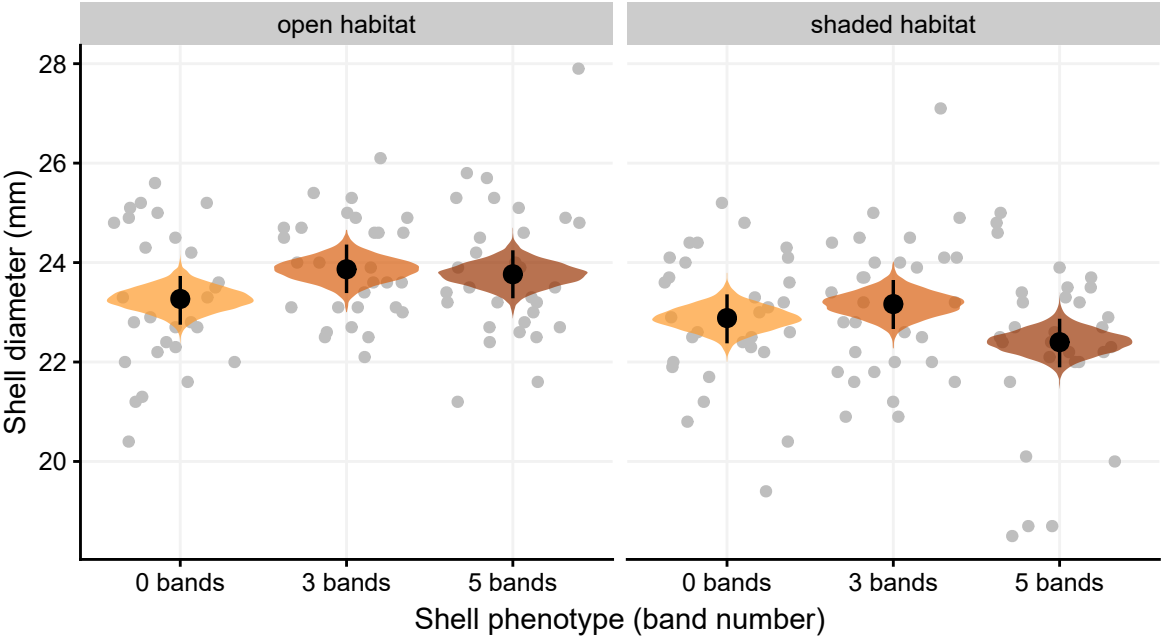
660 Williams, A. & Rae, R. (2016). *Cepaea nemoralis* (Linnaeus, 1758) uses its shell as a defence
661 mechanism to trap and kill parasitic nematodes. *J. Molluscan Stud.* **82**, 349–350.

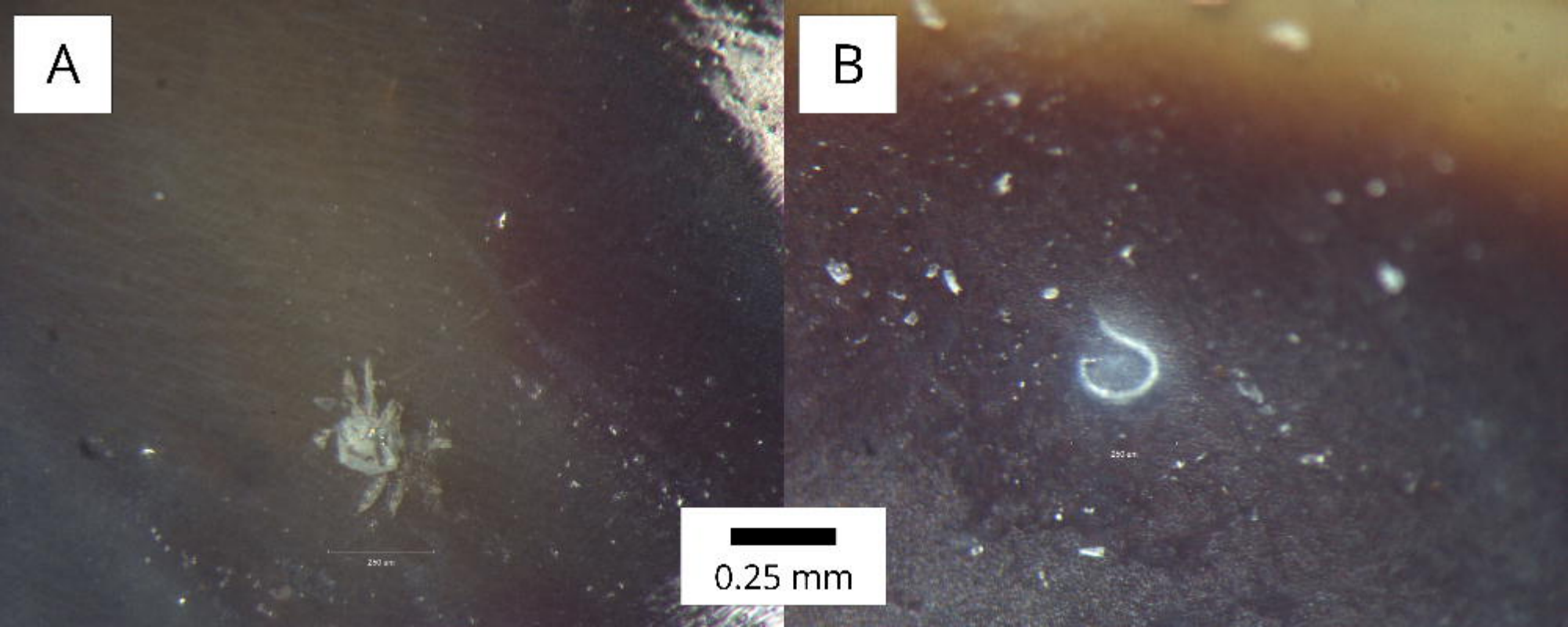
662 Williams, S.T. (2017). Molluscan shell colour. *Biol. Rev.* **92**, 1039–1058.

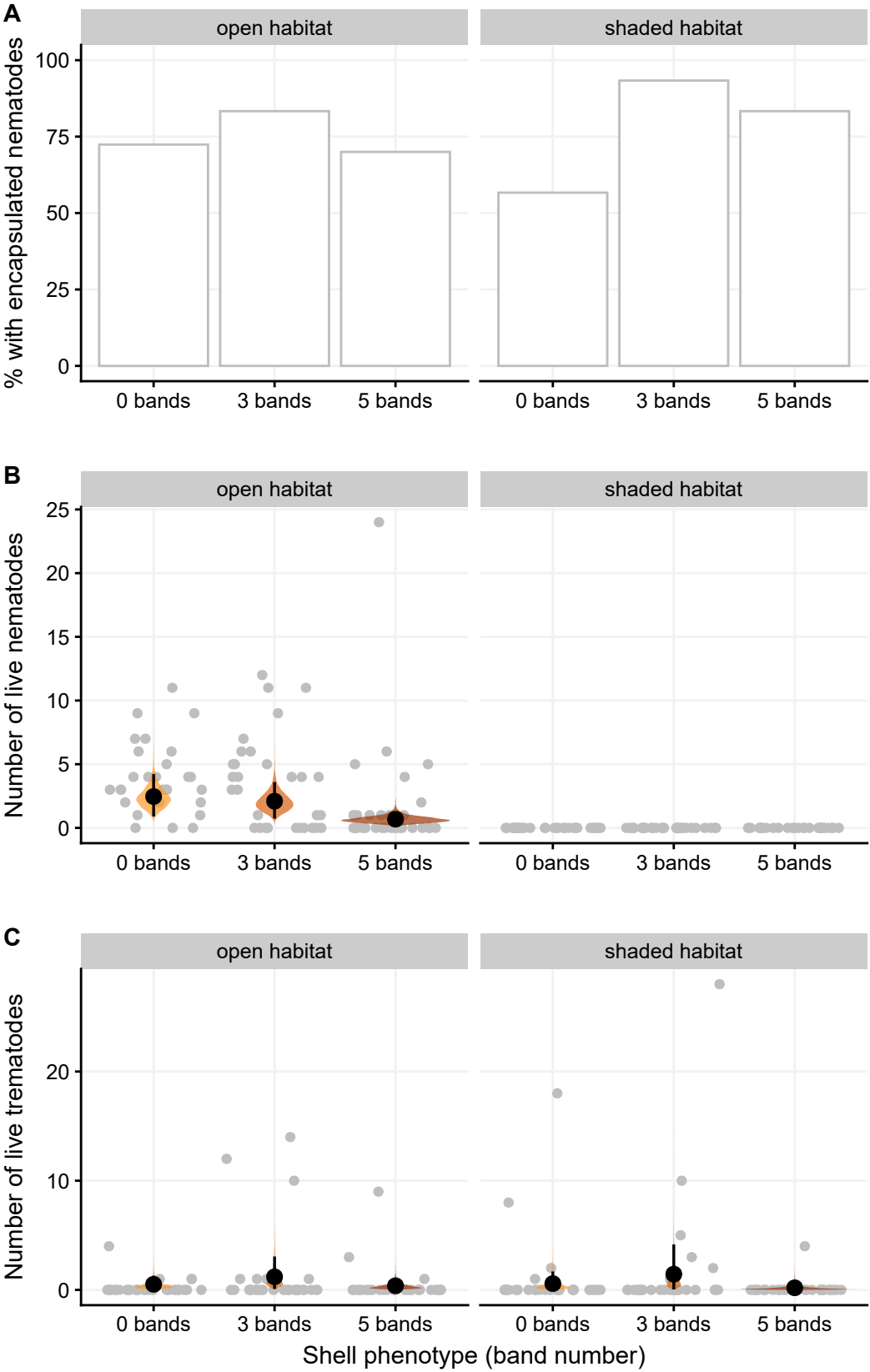
663 Wilson, M.J., Hughes, L.A., Hamacher, G.M. & Glen, D.M. (2000). Effects of *Phasmarhabditis*
664 *hermaphrodita* on non-target molluscs. *Pest. Manag. Sci.* **56**, 711–716.

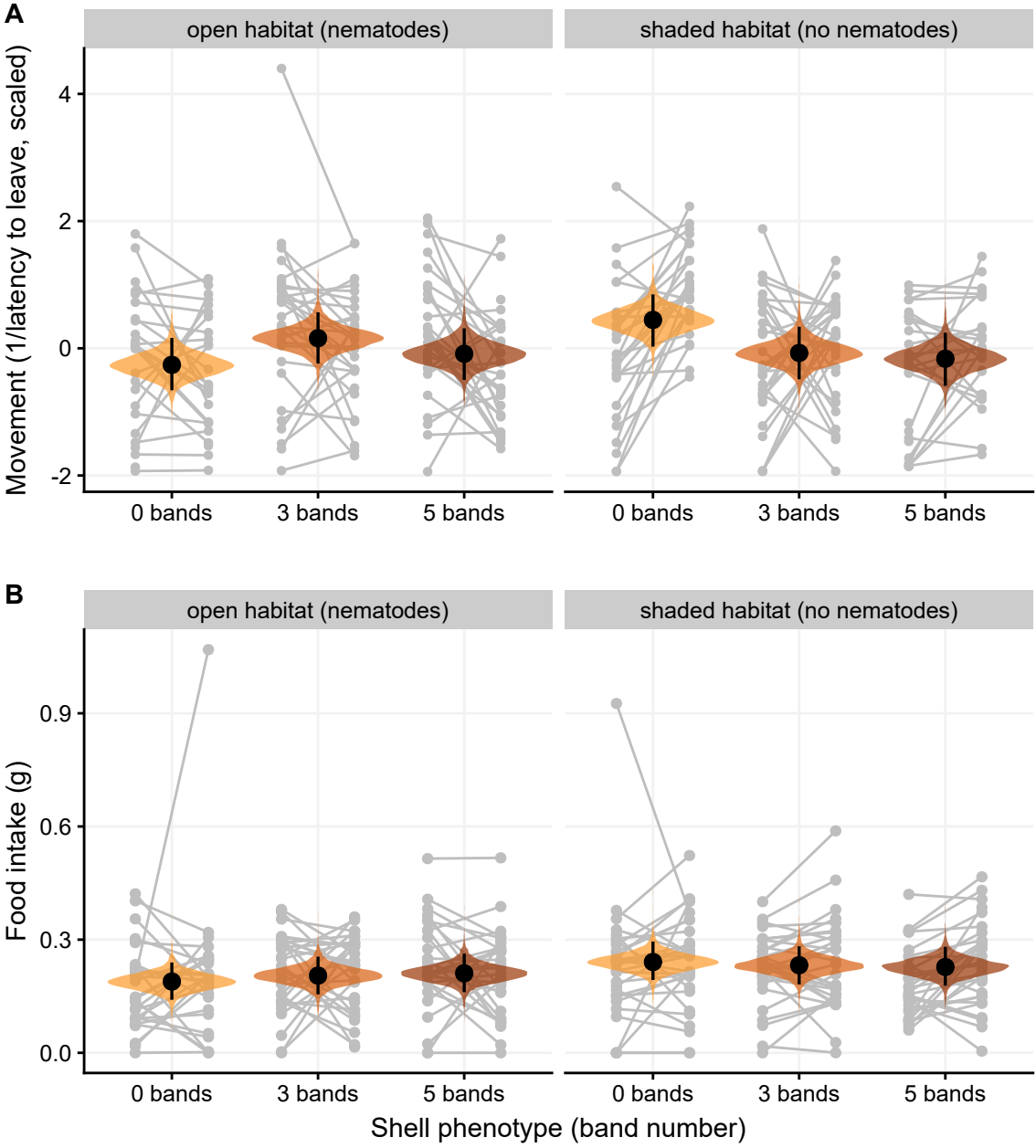
Wynne, R., Morris, A. & Rae, R. (2016). Behavioural avoidance by slugs and snails of the parasitic nematode *Phasmarhabditis hermaphrodita*. *Biocontrol Sci. Technol.* **26**, 1129–1138.

Żbikowska, E., Marszewska, A., Cichy, A., Templin, J., Smorąg, A. & Strzała, T. (2020). *Cepaea* spp. as a source of *Brachylaima mesostoma* (Digenea: Brachylaimidae) and *Brachylecithum* sp. (Digenea: Dicrocoeliidae) larvae in Poland. *Parasitol. Res.* **119**, 145–152.


open habitat
shaded habitat
Shell diameter (mm)
28
26
24
22
20
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
Shell phenotype (band number)


A
B
0.25 mm


A
open habitat
shaded habitat
% with encapsulated nematodes
100
75
50
25
0
0 bands
3 bands
5 bands
B
Number of live nematodes
25
20
15
10
5
C
Number of live trematodes
Shell phenotype (band number)


A
open habitat (nematodes)
shaded habitat (no nematodes)
Movement (1/latency to leave, scaled)
4
2
0
-2
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
B
open habitat (nematodes)
shaded habitat (no nematodes)
Food intake (g)
0.9
0.6
0.3
0.0
0 bands
3 bands
5 bands
0 bands
3 bands
5 bands
Shell phenotype (band number)