

1 Individual boldness is life stage-dependent and linked to dispersal in a hermaphrodite land
2 snail

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4 Maxime Dahirel^{1,2*}, Alexandre Vong², Armelle Ansart², Luc Madec²

5 ¹ Ghent University, Department of Biology, Terrestrial Ecology unit, Gent, Belgium

6 ² Université de Rennes 1/ CNRS, UMR 6553 Ecobio, Rennes, France

7 * corresponding author: maxime.dahirel@yahoo.fr

8 **ABSTRACT**

9 Both individual variation in dispersal tendency and animal personalities have been shown to
10 be widespread in nature. They are often associated in personality-dependent dispersal, and
11 both have major but underappreciated consequences for ecological and evolutionary
12 dynamics. In addition, personalities are not stable over time and changes can appear through
13 ontogeny, leading to life stage-dependent behaviours. We investigated relationships between
14 dispersal, life stage and boldness in an invertebrate with between- and within-life stages
15 variation in dispersal tendency, the land snail *Cornu aspersum*. Latency to exit the shell
16 following a simulated attack was repeatable, indicating boldness is a personality trait in *Cornu*
17 *aspersum*. Subadults were bolder and more dispersive than adults. Dispersers were bolder
18 than non-dispersers, independently of boldness changes between life stages. We discuss how
19 these results can be explained in relation with life history strategies in this hermaphrodite
20 species, in particular risk management in the context of reproductive investment.

21 **Keywords:** age, animal personality, behavioural syndromes, dispersal costs, life histories

22 INTRODUCTION

23 Individual variation in dispersal, *i.e.* in movement leading to gene flow in space, is often
24 correlated with variation in other phenotypic traits (life-history, physiology, morphology,
25 behaviour...)[1–3]. Such “dispersal syndromes” may help dispersing and resident individuals
26 to maximize fitness, offsetting costs incurred during dispersal and in the origin habitat,
27 respectively [1]. Knowledge of these syndromes can yield insights on the proximate and
28 ultimate mechanisms underlying dispersal decisions, and help better appreciate the
29 consequences of dispersal on meta-population functioning [1].

30 Inter-individual differences in behaviour that are consistent across time or contexts (animal
31 personalities) have been described for a large set of taxa and behaviours (although mostly in
32 vertebrates; [4–8] and references therein). They have important yet under-assessed ecological
33 and evolutionary consequences, as individuals exhibiting different personalities also often
34 differ in various other life-history traits [6,8]. Boldness/shyness, broadly defined as an
35 individual’s reaction to risky situations (e.g. predators)[4] is a key personality trait in the
36 context of dispersal [3]. As dispersal presents many costs and risks, bolder individuals are
37 often more dispersive [3]. Dispersal is also often age-structured, with the nature of the most
38 dispersive life stage depending on age-dependent dispersal costs or trade-offs with
39 reproductive investment [9]. While boldness changes through ontogeny have also been
40 documented [10,11], we do not currently know to which extent dispersal-boldness syndromes
41 are linked to age-/life stage-dependent dispersal.

42 Here we analyzed the relationships between boldness, dispersal and life stage in an
43 invertebrate, the land snail *Cornu aspersum* (Müller) (family Helicidae). We tested whether
44 boldness varied with life stage (subadult/ adult) and dispersal status in this species, to
45 determine whether these differences could explain previously documented stage-dependent
46 dispersal patterns [12,13].

47 **MATERIALS AND METHODS**

48 **Snail collection and maintenance**

49 In April 2016, snails were collected by hand in peri-urban parks in Rennes, France. Sixty
50 adults and sixty subadults (greater shell diameter > 20 mm) were used in experiments; adults
51 were recognisable by the presence of a lip around the shell peristome. Snails were
52 individually marked with felt-tip paint markers and maintained under controlled conditions
53 ($20^{\circ}\text{C} \pm 1^{\circ}\text{C}$; 16L: 8D, with scotophase starting at 8:00 pm; *ad libitum* access to cereal flour-
54 based snail food from Hélinove, Le Boupère, France). They were housed by groups of either
55 10 adults or 10 subadults in polyethylene boxes covered by a net ($30 \times 45 \times 8$ cm) and lined
56 by synthetic foam kept saturated with water. Boxes were cleaned and the lining changed once
57 per week. Two snails that died during the experiments were replaced by adding one new
58 individual to two of the following boxes.

59 **Boldness tests**

60 Our protocol is inspired by Seaman and Briffa [7]. Snails were assayed between 16:00 and
61 20:00, i.e. at the end of photophase. Snails were first placed in a Petri dish containing water
62 for up to 5 minutes to stimulate activity, and then placed on glass plates. Once they moved at
63 least 3 cm away from their starting position, an operator used a pipette tip to pinch them for 5
64 seconds on the right side of the foot, close to the peristome. This caused all snails to retract
65 fully within their shells. All tests were carried out by the same operator (A.V.). We used the
66 time snails took to exit their shells following the “attack”, from retraction to the full extension
67 of all tentacles out of the shell, as our measure of boldness (snails with shorter latencies being
68 considered bolder). Two trials, 7 days apart, were conducted on each snail. We tested all
69 snails coming from the same box on the same day, and replaced them in their source box after
70 testing. We stopped tests if a snail had not moved after 20 minutes; these interrupted
71 observations (8 out of 240) were not included in further analyses.

72 **Dispersal tests**

73 Dispersal was assessed in an outdoor tarmacked area with no food or shelter on the Beaulieu
74 university campus, Rennes. Seven days after their second boldness test, snails were placed by
75 life-stage in open boxes, around the middle of the arena for one night (19:00 to 09:00 on the
76 following day). All tests were made on nights with mean temperature > 10 °C and rainfall ≤ 1
77 mm. We tested two boxes per night, one per life stage (adult/ subadult). Boxes tested the same
78 night were separated by at least 6 m, a distance larger than this species' perceptual range [13].
79 No dispersing snail was found in the other box the following morning. Both food and the box
80 lining were left in boxes during tests in order to provide snails with a favourable and familiar
81 environment, and one slate was added in each box to provide shelter. Based on available
82 information on home range and routine movements, only snails recaptured farther than 1 m
83 from the centre of their box were deemed dispersers [12].

84 **Statistical analyses**

85 Analyses were done using R version 3.3.1 and the *lme4* package [14,15]. Differences in
86 dispersal probability between subadults and adults were assessed using a binomial generalized
87 linear mixed model, with a random effect of test night. Log-transformed latencies to resume
88 activity were analysed using a linear mixed model with dispersal status, developmental stage
89 and their interaction as explanatory factors, as well as random effects of individual identity
90 and test session. We used the Satterthwaite approximation as implemented in the *lmerTest*
91 package to determine degrees of freedom for conditional *F* tests [16]. We calculated mixed-
92 model repeatabilities (raw and adjusted for the effects of model variables) and their 95%
93 confidence intervals following Nakagawa and Schielzeth [17] using the *rptR* package.
94 Repeatabilities significantly higher than zero indicate within-individual consistency in
95 behaviour between trials, and were deemed evidence of animal personality [4].

96 **RESULTS**

97 Both raw and adjusted repeatabilities were significantly greater than zero ($R_{raw} = 0.52$, $SE =$
98 0.07 , $95\% \text{ CI} = [0.37, 0.64]$; $R_{adj} = 0.48$, $SE = 0.07$, $95\% \text{ CI} = [0.32, 0.60]$).

99 Subadults were more likely to disperse (Wald test, $X^2 = 4.094$, $p = 0.043$, Fig. 1), and were
100 significantly faster to exit their shells than adults ($F_{1,112.8} = 11.038$, $p = 0.001$; Fig. 2, left).

101 Stage being equal, snails that dispersed were also bolder than those that did not ($F_{1,112.8} =$
102 3.970 ; $p = 0.049$; Fig. 2, right). There was no significant stage \times dispersal status interaction

103 ($F_{1,112.8} = 0.331$, $p = 0.566$).

104 **DISCUSSION**

105 Latency to exit the shell following a stressful stimulus was repeatable in *Cornu aspersum*,
106 even after accounting for two potential confounding variables susceptible of increasing
107 between-individual differences, namely life stage and dispersal status. The present study
108 therefore provides, to our knowledge, the first evidence for animal personality in a terrestrial
109 gastropod, following recent examples in aquatic species [7]. It adds to increasing evidence
110 that animal personalities are widespread, even among non-vertebrates [5].

111 Subadults were more dispersive than adults (Fig. 1), a result in line with previous studies [12]
112 and likely linked to the higher reproductive costs incurred by older snails [18]. Subadults were
113 also bolder than adults (Fig. 2), and dispersers bolder than non-dispersers (Fig. 2). There was
114 no interaction between life stage and dispersal status, meaning that in *Cornu aspersum*,
115 personality-dependent dispersal is consistent across life stages, *i.e.* does not vary whether we
116 consider the least (adult) or the most (subadult) dispersive life stage.

117 Bolder individuals are expected to have a lower survival on average, due to predation for
118 instance [8]. Therefore, observed boldness differences between wild-caught subadults and
119 adults might not reflect true behavioural shifts across ontogeny, but merely be the result of
120 increased mortality of bold subadults, *e.g.* during dispersal [2,8]. While we were not able to
121 separate these two effects here due to the use of wild individuals, true loss of boldness with

122 aging/maturity has been observed in several other species [10,11]. Such behavioural shifts are
123 expected when environmental situations and/ or life history expectations differ between life
124 stages, with for instance later life stages being more risk-averse as a way to protect already
125 acquired resources [11,19]. We expect these differences in life history expectations to play a
126 major role in *Cornu aspersum*: although both stages are able to mate, adults are characterized
127 by a large increase in female reproductive investment [20]. In addition, the same reasoning
128 can be applied to explain the differences in boldness between dispersing and resident snails
129 that were observed even after accounting for the effect of life stage. Indeed, dispersing *C.*
130 *aspersum* snails present lower values of female investment than their more sedentary
131 conspecifics [18]. This suggests a role of neuroendocrine factors associated with reproductive
132 development [21] as common proximate drivers of both boldness and dispersal variation in
133 this species [3].

134 *Cornu aspersum*'s recent history is characterized by serial introductions/ colonizations
135 worldwide [22], and it is often present in highly anthropogenic fragmented environments [13].
136 Although questions remain on how the described boldness-dispersal syndrome changes with
137 environmental context, and on the mechanisms underlying the life-stage dependency of
138 boldness, we provide here some new information on the ecological consequences of
139 individual personalities in an invertebrate species. Given the predicted role of personality-
140 dependent dispersal in biological invasions and metapopulation dynamics [3], our results may
141 help shed light on the mechanisms behind this species' worldwide success.

142 **DATA ACCESSIBILITY**

143 Data will be uploaded to Dryad or similar repositories upon acceptance.

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146 **AUTHORS' CONTRIBUTIONS**

147 M.D., A.A. and L.M. conceived the study and designed the experiments. A.V. carried out the
148 experiments. M.D. and A.V. conducted analyses and drafted the manuscript, with input from
149 all authors. All authors gave final approval for publication.

150 **COMPETING INTERESTS**

151 The authors declare no competing interests.

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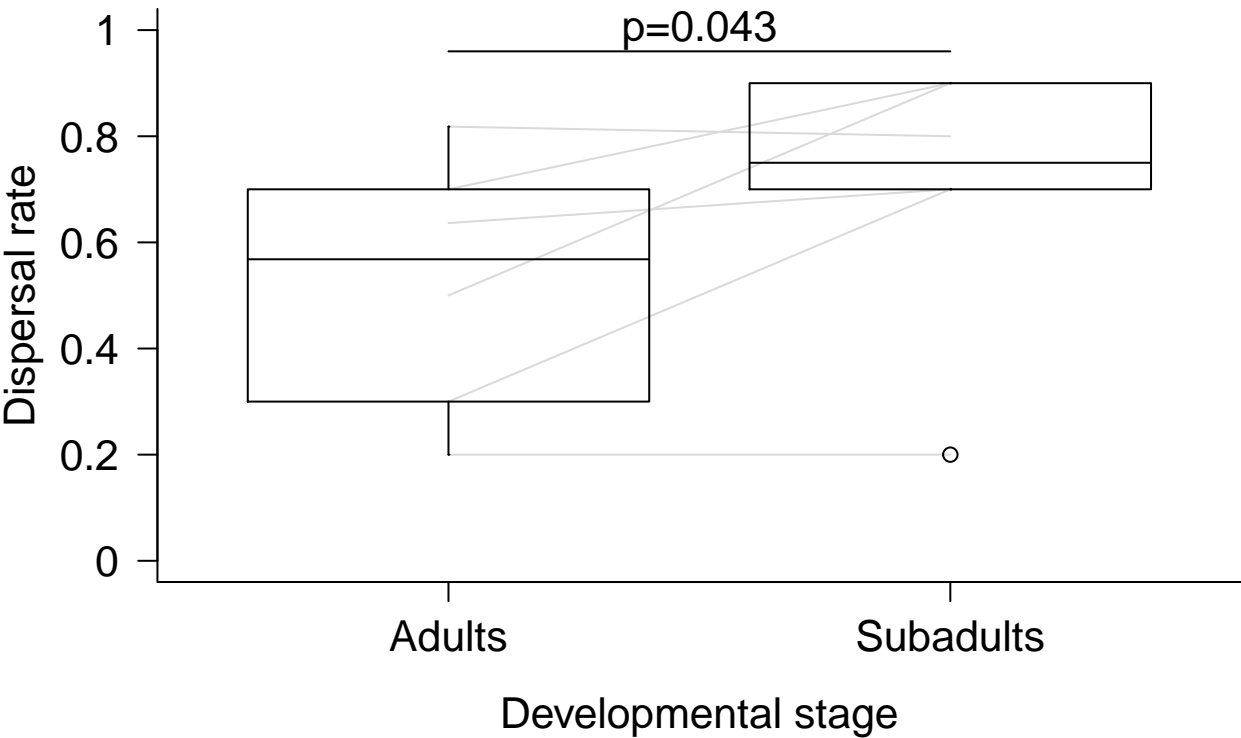
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213 **FIGURES LEGENDS**

214 **Figure 1.** Dispersal rate per test box as a function of developmental stage. *P* value is based on
215 a binomial generalized linear mixed model. Grey lines connect observed values of boxes
216 tested on the same night.

217 **Figure 2.** Stage- (left) and dispersal- (right) related differences in boldness (latency to exit
218 shell). *P* values are based on a linear mixed model. Model was fitted, and data are plotted, on
219 the log scale. Grey lines connect values from the same individual.



shy



bold

