

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

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## 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  $\leq 1$   
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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213 **FIGURES LEGENDS**

214 **Figure 1.** Dispersal rate per test box as a function of developmental stage. *P* value is based on

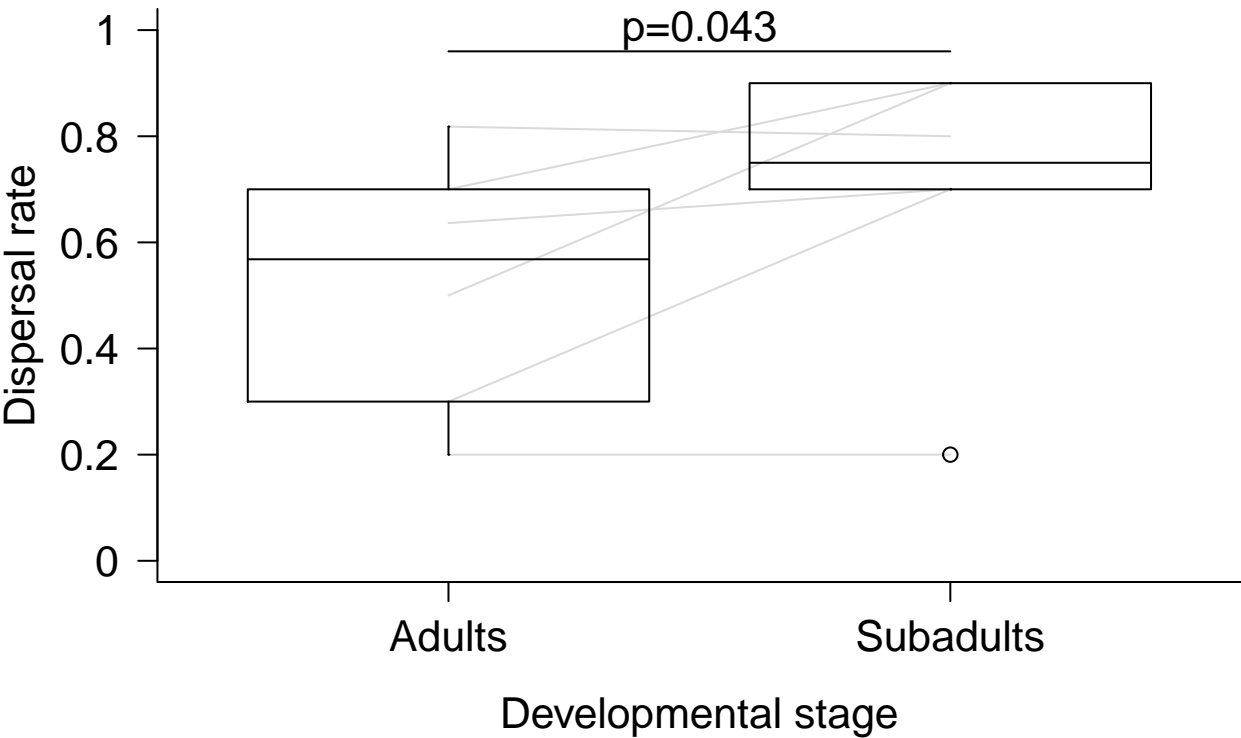
215 a binomial generalized linear mixed model. Grey lines connect observed values of boxes

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

