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 $> 10^{\circ}\text{C}$ 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**

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

Subadults were more likely to disperse (Wald test, $X^2 = 4.094$, $p = 0.043$, Fig. 1), and were significantly faster to exit their shells than adults ($F_{1,112.8} = 11.038$, $p = 0.001$; Fig. 2, left). Stage being equal, snails that dispersed were also bolder than those that did not ($F_{1,112.8} = 3.970$; $p = 0.049$; Fig. 2, right). There was no significant stage $\times$ dispersal status interaction ($F_{1,112.8} = 0.331$, $p = 0.566$).

DISCUSSION

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

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

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

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

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

DATA ACCESSIBILITY

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

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AUTHORS' CONTRIBUTIONS

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

COMPETING INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Ronce, O. & Clobert, J. 2012 Dispersal syndromes. In *Dispersal ecology and evolution* (eds J. Clobert M. Baguette T. G. Benton & J. M. Bullock), pp. 119–138. Oxford, UK: Oxford University Press.
2. Bonte, D. et al. 2012 Costs of dispersal. *Biol. Rev.* **87**, 290–312. (doi:10.1111/j.1469-185X.2011.00201.x)
3. Cote, J., Clobert, J., Brodin, T., Fogarty, S. & Sih, A. 2010 Personality-dependent dispersal: characterization, ontogeny and consequences for spatially structured populations. *Philos. Trans. R. Soc. B Biol. Sci.* **365**, 4065–4076. (doi:10.1098/rstb.2010.0176)
4. Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007 Integrating animal temperament within ecology and evolution. *Biol. Rev.* **82**, 291–318. (doi:10.1111/j.1469-185X.2007.00010.x)
5. Kralj-Fišer, S. & Schuett, W. 2014 Studying personality variation in invertebrates: why bother? *Anim. Behav.* **91**, 41–52.
6. Wolf, M. & Weissing, F. J. 2012 Animal personalities: consequences for ecology and evolution. *Trends Ecol. Evol.* **27**, 452–461. (doi:10.1016/j.tree.2012.05.001)

- 170 7. Seaman, B. & Briffa, M. 2015 Parasites and personality in periwinkles (*Littorina littorea*):
171 Infection status is associated with mean-level boldness but not repeatability. *Behav.*
172 *Processes* **115**, 132–134. (doi:10.1016/j.beproc.2015.03.014)
- 173 8. Smith, B. R. & Blumstein, D. T. 2008 Fitness consequences of personality: a meta-
174 analysis. *Behav. Ecol.* **19**, 448–455. (doi:10.1093/beheco/arm144)
- 175 9. Benton, T. G. & Bowler, D. E. 2012 Dispersal in invertebrates: influences on individual
176 decisions. In *Dispersal ecology and evolution* (eds J. Clobert M. Baguette T. G. Benton &
177 J. M. Bullock), pp. 41–49. Oxford, UK: Oxford University Press.
- 178 10. Niemelä, P. T., Vainikka, A., Hedrick, A. V. & Kortet, R. 2012 Integrating behaviour with
179 life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Funct. Ecol.* **26**,
180 450–456. (doi:10.1111/j.1365-2435.2011.01939.x)
- 181 11. Gyuris, E., Feró, O. & Barta, Z. 2012 Personality traits across ontogeny in firebugs,
182 *Pyrrhocoris apterus*. *Anim. Behav.* **84**, 103–109. (doi:10.1016/j.anbehav.2012.04.014)
- 183 12. Dahirel, M., Vardakis, M., Ansart, A. & Madec, L. 2016 Density-dependence across
184 dispersal stages in a hermaphrodite land snail: insights from discrete choice models.
185 *Oecologia* **181**, 1117–1128. (doi:10.1007/s00442-016-3636-z)
- 186 13. Dahirel, M., Séguret, A., Ansart, A. & Madec, L. 2016 Dispersal-related traits of the snail
187 *Cornu aspersum* along an urbanisation gradient: maintenance of mobility across life stages
188 despite high costs. *Urban Ecosyst.* , 1–13. (doi:10.1007/s11252-016-0564-y)
- 189 14. R Core Team 2016 *R: a language and environment for statistical computing*. Vienna,
190 Austria: R Foundation for Statistical Computing.

15. Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015 Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**. (doi: 10.18637/jss.v067.i01)
16. Luke, S. G. 2016 Evaluating significance in linear mixed-effects models in R. *Behav. Res. Methods*, 1–9. (doi:10.3758/s13428-016-0809-y)
17. Nakagawa, S. & Schielzeth, H. 2010 Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.* **85**, 935–956. (doi:10.1111/j.1469-185X.2010.00141.x)
18. Dahirel, M., Ansart, A. & Madec, L. 2016 Potential syndromes linking dispersal and reproduction in the hermaphrodite land snail *Cornu aspersum*. *J. Zool.* **299**, 98–105. (doi:10.1111/jzo.12328)
19. Wolf, M., van Doorn, G. S., Leimar, O. & Weissing, F. J. 2007 Life-history trade-offs favour the evolution of animal personalities. *Nature* **447**, 581–584. (doi:10.1038/nature05835)
20. Bride, J. & Gomot, L. 1991 Asynchronisme du développement du tractus génital de l'escargot *Helix aspersa* pendant la croissance et la reproduction. *Reprod. Nutr. Dev.* **31**, 81–96.
21. Gomot de Vaufléury, A. 2001 Regulation of growth and reproduction. In *The biology of terrestrial molluscs* (ed G. M. Barker), pp. 331–355. Wallingford, UK: CABI.
22. Guiller, A., Martin, M.-C., Hiraux, C. & Madec, L. 2012 Tracing the invasion of the mediterranean land snail *Cornu aspersum aspersum* becoming an agricultural and garden pest in areas recently introduced. *PLoS ONE* **7**, e49674. (doi:10.1371/journal.pone.0049674)

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