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Author for correspondence:
Johan Ahlgren
e-mail: johan.ahlgren@biol.lu.se

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Animal behaviour

Individual boldness is linked to protective shell shape in aquatic snails

Johan Ahlgren[1], Ben B. Chapman[1,2], P. Anders Nilsson[1,3] and Christer Brönmark[1]

[1]Department of Biology-Aquatic Ecology, Lund University, Ecology Building, 22362 Lund, Sweden
[2]School of Life Sciences, University of Nottingham, Nottingham, UK
[3]Department of Environmental and Life Sciences-Biology, Karlstad University, 65188 Karlstad, Sweden

JA, 0000-0002-6506-289X

The existence of consistent individual differences in behaviour ('animal personality') has been well documented in recent years. However, how such individual variation in behaviour is maintained over evolutionary time is an ongoing conundrum. A well-studied axis of animal personality is individual variation along a bold–shy continuum, where individuals differ consistently in their propensity to take risks. A predation-risk cost to boldness is often assumed, but also that the reproductive benefits associated with boldness lead to equivalent fitness outcomes between bold and shy individuals over a lifetime. However, an alternative or complementary explanation may be that bold individuals phenotypically compensate for their risky lifestyle to reduce predation costs, for instance by investing in more pronounced morphological defences. Here, we investigate the 'phenotypic compensation' hypothesis, i.e. that bold individuals exhibit more pronounced anti-predator defences than shy individuals, by relating shell shape in the aquatic snail *Radix balthica* to an index of individual boldness. Our analyses find a strong relationship between risk-taking propensity and shell shape in this species, with bolder individuals exhibiting a more defended shell shape than shy individuals. We suggest that this supports the 'phenotypic compensation' hypothesis and sheds light on a previously poorly studied mechanism to promote the maintenance of personality variation among animals.

1. Introduction

Individual animals within populations can differ consistently in behavioural traits, across contexts and time. These consistent behaviours are often called animal personalities, temperaments or behavioural types, and have been documented in a wide range of behavioural traits associated with activity [1], anti-predator [2], exploration [3] and risk-taking [4]. Of these, individual variation along the bold/shy continuum [5] has received most attention. This continuum ranges from bold individuals that consistently engage in risky behaviours, to shy individuals that are consistently more unwilling to take risks [6]. The different lifestyles of bold and shy individuals are likely to have fitness consequences: for example, bold individuals have been found to have higher foraging rates [7] and more mating opportunities [8], but may also experience a higher mortality rate from predation [9]. However, the pros and cons of these different lifestyles seem to be species- and context-dependent, as other studies have shown lower mortality in bold individuals [10,11]. As yet, few studies evaluate how bold individuals trade off safety from predators for foraging and reproductive benefits. One explanation, which is largely untested, is that bold individuals may be able to compensate for an increased susceptibility to predation by expressing alternative phenotypic traits that reduce risk, the 'phenotypic compensation' hypothesis. There are several examples of potential

trait compensation in the literature, involving behavioural and morphological defences [12], but only a handful of studies have related personality types to the differential expression of defence traits. For example, bold individuals of the lizard *Anolis sagrei* need less force to autotomize their tail when attacked by predators [13]. Furthermore, bold crucian carp induce a more pronounced morphological defence when exposed to predators which reduces their vulnerability to gape-limited piscivores and may thus compensate for their risky lifestyle [14]. If the phenotypic compensation hypothesis has broad explanatory power, then we should predict bold individuals to exhibit more pronounced defences than shy individuals, to ameliorate their increased susceptibility to predation. Yet, to the best of our knowledge few, if any, studies have addressed how standing variation in boldness may be linked to defensive morphology.

In this study, we investigate whether bold individuals of the freshwater snail *Radix balthica* exhibit phenotypic compensation by investing in morphological defence traits that reduce predation mortality. In this and many other snail species, shell shape is highly variable at an intraspecific level and, a number of studies have related shell shape to predation vulnerability [15]. To test our hypothesis that increased boldness correlates with a more defended shell phenotype, we collected snails from four different ponds, and quantified boldness and shell shape for individuals. We predicted that owing to the risky lifestyle of bold individuals they should have rounder and, hence, more protective shells than shy snails.

2. Material and methods

(a) Study organisms

Egg capsules of the freshwater snail *R. balthica* were collected from four fish-free ponds close to Lund, Sweden, brought to the laboratory, held separated in 2 l aquarium and fed *Spirulina* and lettuce ad libitum (see electronic supplementary material for more details). Three months after hatching the juvenile, snails were individually tagged with numbered bee tags [16] and were then left for at least 48 h before the first behavioural assay. We chose snails from fish-free ponds because they have a wider distribution of snail boldness with both bold and shy individuals present [10].

(b) Personality assessment

To quantify the personality type of individual snails, we used the time to emergence from a refuge. Each snail was allowed to retract into its shell, its own refuge, in response to a fright stimulus [17,18]. The assay was repeated after one week (see electronic supplementary material for more detail). Although boldness is a continuous trait, snails were categorized as either bold or shy to enable effective assessment of the impact of boldness upon shell shape in a factorial design, an approach adopted in previous studies [14]. Our bold snails had an emergence time less than or equal to 10 s in both trials as bold, whereas shy snails had an emergence time greater than or equal to 15 s in both trials. In total, 168 snails were assessed, out of which 160 (95.2% of snails assessed) matched our categories and were hence included in the morphological analysis, 76 bold (mean emergence time: 5.23 ± 1.77 s) and 84 shy (mean emergence time: 57.66 ± 30.14 s).

(c) Shell shape assessments

After the behavioural assessments, snails were placed with their aperture facing downwards on a flatbed scanner (Epson 2450 Photo) together with a ruler (10×10 mm) for shape measurements. Images were then analysed in SHAPE [19], which generates principal components (PCs) explaining the shape variation in the outline of the shells.

(d) Statistical analysis

To test for individual consistency in the personality trait emergence time/boldness, we used Spearman's rank correlation and a repeatability analysis (see electronic supplementary material). We assessed differences in shell shape between personality types with nested ANOVA, where factor 'egg capsule' was nested within the factor 'pond origin' to allow the use of individual snails as replicates. All statistical analyses were performed in R 3.1.0 [20], using the ggplot package to produce the density plots [21].

3. Results

Snail boldness (time to emergence) showed high individual consistency (Spearman's $n = 168$, $\rho = 0.844$, $p < 0.001$) and repeatability ($r = 0.64$) between trials (electronic supplementary material, figure S1). The two first PCs made up 80% of the shell shape variation with PC1 explaining 66% and PC2 14%. In the first PC, positive values were associated with round shells with a wider body whorl and a larger aperture, whereas negative values indicated a narrower aperture and a longer and well-defined apex (figure 1). In the second PC, positive values were associated with a widening of the aperture and narrowing of the second to last whorl, whereas the opposite was true for negative values. There was a significant difference between bold and shy snails in shell shape as defined by PC1 (ANOVA, $F_{1,18} = 10.7$, $p = 0.004$), with bold snails having positive PC1 scores and thereby a rounder shell shape (figure 2*a*). There was also a significant difference between bold and shy snails in shell shape as defined by PC2 (ANOVA, $F_{1,18} = 4.5$, $p = 0.048$) with bold snails having a wider aperture than shy snails (figure 2*b*). Also, factor 'egg capsule' nested within factor 'pond origin' was significant for both PC1 and PC2 (ANOVA, PC1; $F_{18,140} = 23.4$, $p < 0.001$, PC2; $F_{18,140} = 4.1$, $p < 0.001$).

4. Discussion

Although studies on animal personalities are increasing in frequency, it is still difficult to make general predictions of fitness-related costs and benefits of different personality types, and the data are currently equivocal. While some studies report that bold and active individuals suffer an increased susceptibility to predation [9], high predation pressure can actually sometimes favour boldness [11]. With regards to our study organism *R. balthica*, we recently showed that bold individuals have a higher survival than shy snails when experimentally exposed to fish predation and, further, that snails from ponds with fish predators were all bold, whereas snails from fish-free ponds had a wider distribution of snail boldness with both bold and shy individuals present [10]. At first, it seems a paradox that bold snails with a more risky lifestyle are dominant in high-risk habitats like fish ponds. The data we present here may provide an explanation for this and similar patterns. In the light of the phenotypic compensation hypothesis, we confirm that snail individuals show consistent boldness traits (emergence time), and that bold snails have significantly

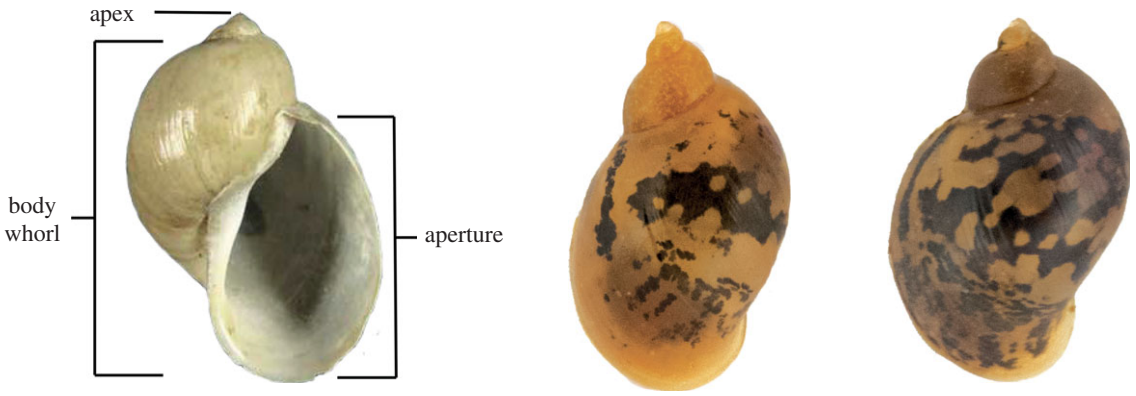

Figure 1. (Left) A shell describing the morphological characters, examples of an elongated and less protected shell and a rounder and thicker shell with a wider aperture (right). (Online version in colour.)

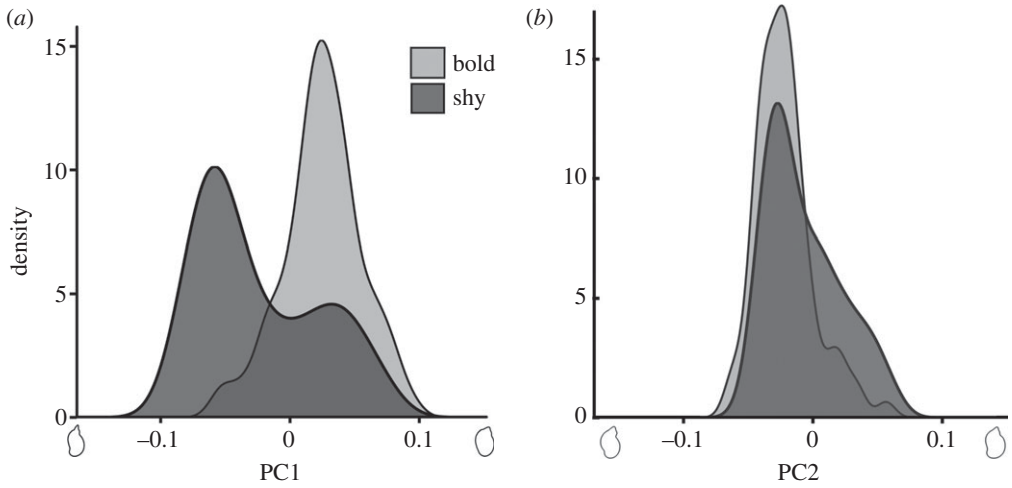

Figure 2. (*a*,*b*) Density plots of the shell shape in bold and shy *Radix balthica*. Shell shape characteristics are expressed as principal component scores (*a*) (PC1) and (*b*) (PC2) with the visual shapes under the *x*-axis.

more rounded shells and a wider aperture, hence more defended shells than shy individuals. Morphological defences are an extremely common feature in the animal kingdom and they can both lower encounter rates [22] as well as increase prey survival upon an encounter [23]. In the freshwater snail *R. balthica*, rounder shells with wider apertures have been shown to increase crushing resistance, thereby reducing vulnerability to shell-crushing predators like fish [24]. These protective shells, however, come with a cost [25]. If bolder individuals are at greater risk of predation owing to their risky behaviour, natural selection for a more defended shell phenotype may be stronger than for risk-averse individuals, resulting in the covariation between shell shape and personality we report here. Alternatively, having more effective defensive structures may allow animals to be bolder: hermit crabs (*Pagurus bernhardus*) are bolder when better camouflaged [26]. The correlation between shell shape and boldness could be explained by phenotypic compensation, although an important next step to confirm this would be to estimate fitness consequences for bold and shy snails with different shell shapes when exposed to predators. Phenotypic compensation in bold individuals is not well studied as yet, but a few examples from the literature support this hypothesis in varying situations and with different compensatory traits [13,14]. Furthermore, it has been shown that fish predators have a strong selection pressure on both boldness [10] and shell shape in our study species [27]. Interestingly, our snails were collected from fish-free ponds and reared in a common-garden situation in the absence of predators, suggesting that the covariation between boldness and shell shape is genetically determined, and not an effect of phenotypically plastic responses to predation. It is hence possible that selection and the ghost of predation past reveal themselves as bold snails having more protective shells even in fish-free systems.

Ethics statement. Swedish authorities do not require ethical permissions for research on invertebrate species.

Data accessibility. The data underlying this study are available on Dryad: doi:10.5061/dryad.dj827.

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Authors' contributions. J.A. and B.B.C. came up with the idea. J.A. developed the experimental design together with B.B.C., C.B. and A.N. J.A. performed the experiment. J.A. did the statistical analysis and prepared figures with help from A.N. J.A. wrote the paper together with B.B.C., C.B. and A.N. All authors gave final approval for publication.

Conflict of interest. We have no competing interests.

References

1. Kurvers RH, Eijkelenkamp B, van Oers K, van Lith B, van Wieren SE, Ydenberg RC, Prins HH. 2009 Personality differences explain leadership in barnacle geese. *Anim. Behav.* **78**, 447–453. (doi:10.1016/j.anbehav.2009.06.002)
2. Quinn JL, Cresswell W. 2005 Personality, anti-predation behaviour and behavioural plasticity in the chaffinch *Fringilla coelebs*. *Behaviour* **142**, 1377–1402. (doi:10.2307/4536305)
3. Minderman J, Reid JM, Evans PG, Whittingham MJ. 2009 Personality traits in wild starlings: exploration behavior and environmental sensitivity. *Behav. Ecol.* **4**, 830–837. (doi:10.1093/beheco/arp067)
4. van Oers K, Klunder M, Drent PJ. 2005 Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. *Behav. Ecol.* **16**, 716–723. (doi:10.1093/beheco/ari045)
5. Ward AJ, Thomas P, Hart PJ, Krause J. 2004 Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.* **55**, 561–568. (doi:10.1007/s00265-003-0751-8)
6. Wilson DS, Clark AB, Coleman K, Dearstyne T. 1994 Shyness and boldness in humans and other animals. *Trends Ecol. Evol.* **9**, 442–446. (doi:10.1016/0169-5347(94)90134-1)
7. Ioannou CC, Payne M, Krause J. 2008 Ecological consequences of the bold–shy continuum: the effect of predator boldness on prey risk. *Oecologia* **157**, 177–182. (doi:10.1007/s00442-008-1058-2)
8. Reaney LT, Backwell PRY. 2007 Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behav. Ecol.* **18**, 521–525. (doi:10.1093/beheco/arm014)
9. Biro PA, Abrahams MV, Post JR, Parkinson EA. 2004 Predators select against high growth rates and risk-taking behaviour in domestic trout populations. *Proc. R. Soc. Lond. B* **271**, 2233–2237. (doi:10.1098/rspb.2004.2861)
10. Ahlgren J, Kinnby A, Chapman BB, Nilsson PA, Brönmark C. Submitted. Fish favour the bold: predator regimes drive survival and distribution of animal behavioural types.
11. Réale D, Festa-Bianchet M. 2003 Predator-induced natural selection on temperament in bighorn ewes. *Anim. Behav.* **65**, 463–470. (doi:10.1006/anbe.2003.2100)
12. Brodie ED. 1989 Genetic correlations between morphology and antipredator behaviour in natural populations of the garter snake *Thamnophis ordinoides*. *Nature* **342**, 542–543. (doi:10.1038/342542a0)
13. Kuo C-Y, Irschick DJ, Lailvaux SP. 2014 Trait compensation between boldness and the propensity for tail autotomy under different food availabilities in similarly aged brown anole lizards. *Funct. Ecol.* **29**, 385–392. (doi:10.1111/1365-2435.12324)
14. Hulthén K, Chapman BB, Nilsson PA, Hollander J, Brönmark C. 2014 Express yourself: bold individuals induce enhanced morphological defences. *Proc. R. Soc. B* **281**, 20132703. (doi:10.1098/rspb.2013.2703)
15. Hoverman JT, Relyea RA. 2009 Survival trade-offs associated with inducible defences in snails: the roles of multiple predators and developmental plasticity. *Funct. Ecol.* **23**, 1179–1188. (doi:10.1111/j.1365-2435.2009.01586.x).
16. Henry P-Y, Jarne P. 2007 Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. *Invert. Biol.* **126**, 138–153. (doi:10.1111/j.1744-7410.2007.00084.x)
17. Orr MV, El-Bekai M, Lui M, Watson K, Lukowiak K. 2007 Predator detection in *Lymnaea stagnalis*. *J. Exp. Biol.* **210**, 4150–4158. (doi:10.1242/jeb.010173)
18. Briffa M, Rundle SD, Fryer A. 2008 Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proc. R. Soc. B* **275**, 1305–1311. (doi:10.1098/rspb.2008.0025)
19. Iwata H, Ukai Y. 2002 SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. *J. Hered.* **93**, 384–385. (doi:10.1093/jhered/93.5.384)
20. R Development Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical.
21. Wickham H. 2009 *ggplot2: elegant graphics for data analysis*. New York, NY: Springer. See http://had.co.nz/ggplot2/book.
22. Ryer CH, Lemke JL, Boersma K, Levas S. 2008 Adaptive coloration, behavior and predation vulnerability in three juvenile north Pacific flatfishes. *J. Exp. Mar. Biol. Ecol.* **359**, 62–66. (doi:10.1016/j.jembe.2008.02.017)
23. Nilsson PA, Brönmark C, Pettersson L. 1995 Benefits of a predator-induced morphology in crucian carp. *Oecologia* **104**, 291–296. (doi:10.1007/BF00328363)
24. Lakowitz T, Brönmark C, Nyström PER. 2008 Tuning in to multiple predators: conflicting demands for shell morphology in a freshwater snail. *Freshw. Biol.* **53**, 2184–2191. (doi:10.1111/j.1365-2427.2008.02045.x)
25. Brönmark C, Lakowitz T, Nilsson PA, Ahlgren J, Lennartsdotter C, Hollander J. 2012 Costs of inducible defence along a resource gradient. *PLoS ONE* **7**, e30467. (doi:10.1371/journal.pone.0030467)
26. Briffa M, Twyman C. 2011 Do I stand out or blend in? Conspicuousness awareness and consistent behavioural differences in hermit crabs. *Biol. Lett.* **7**, 330–332. (doi:10.1098/rsbl.2010.0761)
27. Brönmark C, Lakowitz T, Hollander J. 2011 Predator-induced morphological plasticity across local populations of a freshwater snail. *PLoS ONE* **6**, e21773. (doi:10.1371/journal.pone.0021773)