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Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator

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Animals with highly inducible traits may show no inducible response when exposed to a related but wholly novel cue. This appears to be true for the intertidal whelk *Nucella lamellosa* faced with a voracious introduced predator. In the laboratory, we exposed whelks to effluent from two species of predatory crab, the native red rock crab *Cancer productus* and the invasive European green crab *Carcinus maenas*. *Nucella* and *Cancer* have a long shared history in the northeast Pacific, whereas potential interaction with *Carcinus* began here less than 10 years ago. Although *Nucella* responded adaptively to *Cancer* effluent by increasing shell thickness and decreasing somatic growth, there was no such response to *Carcinus*. Furthermore, thicker shelled *Nucella* were less likely to be eaten by *Carcinus*. Because *Nucella* produces thicker shells when exposed to *Cancer* cues, its ability to respond similarly to *Carcinus* depends only on the coupling of the *Carcinus* cue to the existing developmental pathways for adaptive changes in shell form. Such coupling of latent plasticity to a novel cue—via genetic changes or associative learning—could explain many cases of rapid phenotypic change following a sudden shift in the environment.

Keywords: inducible defence; phenotypic plasticity; invasive species

1. INTRODUCTION

Though predator-induced defences are well documented (Schlichting & Pigliucci 1998; Tollrian & Harvell 1999; Agrawal 2001; West-Eberhard 2005), we remain largely ignorant about the mechanisms that underlie their origin and, by extension, evolution (but see Crispo 2007). Here, we parse inducible defences into two components: (i) developmental factors that govern phenotypic flexibility, i.e. the capacity for multiple developmental trajectories and (ii) the ability to detect and properly interpret environmental risk cues that trigger adaptive changes to phenotype. Importantly, when developmental flexibility and cue recognition are not coupled, developmental plasticity will not be realized (i.e. latent).

In the northeast Pacific, *Nucella lamellosa* (an intertidal whelk) and *Cancer productus* (a molluscivorous crab) have lived sympatrically since at least the last glacial maximum. By contrast, *N. lamellosa* and another voracious predator, the European green crab *Carcinus maenas*, have coexisted here for less than 10 years (Gillespie *et al.* 2007). Although ranges of *Nucella* and *Carcinus* currently overlap from central California to Vancouver Island, *Carcinus* is likely to continue invading northward, perhaps as far as Alaska where temperature conditions may be comparable to parts of its native range (e.g. Norway). Thus, in time, *Carcinus* may inhabit a sizeable portion of *Nucella*'s native range from central California to the Bering Strait. Because *Nucella* produces thicker shells when exposed to *Cancer* cues (Appleton & Palmer 1988; Marko & Palmer 1991), its ability to respond similarly to *Carcinus* depends only on the coupling of the *Carcinus* cue to the existing developmental pathways for adaptive changes in shell form. Therefore, this ongoing invasion by *Carcinus* presents a rare opportunity to study the early evolution of developmental plasticity in prey in response to a novel predator.

We tested the latent plasticity hypothesis—that the machinery for adaptive phenotypic plasticity is present but not coupled with novel risk cue recognition—by comparing developmental flexibility in *Nucella* exposed to water-borne cues from *Cancer* versus *Carcinus* crabs. Furthermore, we tested whether predator-induced changes in shell form were adaptive by feeding *Nucella* from the induction experiment directly to *Carcinus* in the laboratory.

2. MATERIAL AND METHODS

In October 2007, male European green crabs *C. maenas* were collected from Toquart Bay (49°19.5′ N, 125°19.4′ W), male red rock crabs *C. productus* were collected from Grappler Inlet (48°49.8′ N, 125°07.1′ W) and *N. lamellosa* whelks (shell breadth = 7.92–15.19 mm) were collected from Wizard Islet (48°51.5′ N, 125°09.5′ W) in Barkley Sound, Canada. Significantly, *Carcinus* have not yet invaded Wizard Islet; therefore these snails were naive with respect to *Carcinus*-specific cues. Snails were individually tagged, measured and placed—along with barnacles for food—in 12 × 12 × 8 cm plastic containers with perforated walls. After acclimation to laboratory conditions (15 days), six treatments were administered for 50 days: effluent from (i) large (83–104 mm carapace width, CW) or (ii) small (46–70 mm CW) *Cancer*, effluent from (iii) large (80–94 mm CW) or (iv) small (62–70 mm CW) *Carcinus*, (v) low food, and (vi) controls. Large and small crabs were used to alter the amount of risk cues present, assuming cue scales with crab biomass. Each treatment replicate ($n = 4$) consisted of a treatment chamber (e.g. containing one crab or no crab as control) connected to a snail chamber containing four snails. Seawater flowed from treatment chambers to snail chambers at approximately 1 l min^{-1}. All snails were fed barnacles (*Balanus glandula*) *ad libitum*, except for snails in the low-food treatment, which were offered barnacles for 24 hours every 10th day of the experiment. Crabs were fed once per week with approximately 4.5 g of fish (flounder). Control and low-food snails were exposed to chopped fish effluent on crab feeding days.

Shell lip thickness was measured to the nearest 0.01 mm with dial calipers at the middle of the apertural lip and at the lip suture, and averaged. (Blind remeasurement of 24 shells indicated an 8.5% measurement error for lip thickness.) Tissue mass was measured non-destructively as the difference between whole animal air dry weight and whole animal underwater weight, following Palmer (1982). Somatic growth (mg d^{-1}) was calculated as the tissue mass gained divided by the duration of treatments (50 days).

We used nested ANCOVA to test for differences in somatic growth rate and shell thickness among treatments. The treatment was a fixed categorical effect and container nested within treatment was a random effect. Initial tissue mass was a covariate in analysis for differences in somatic growth. Shell breadth and tissue growth rate were included as covariates when testing for differences in shell thickness because both can contribute to shell thickness variation. All models conformed to assumptions of residual normality ($p > 0.053$; Shapiro–Wilks) and equal variances ($p > 0.07$; Levene's).

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When appropriate, higher order interaction terms were sequentially removed following Hendrix *et al.* (1982). Where treatment effects were significant, *post hoc* Tukey's HSD was used to test for differences among all pairs of least-squares means.

To test whether induced changes in shell form were adaptive, experimental snails were offered directly to European green crabs (*Carcinus*). Six crabs, ranging in carapace width from 58 to 95 mm, were each caged with 12 snails (two from each of the six treatments). Snails were marked so that post-predation shell fragments could be identified, and shell fragments from killed snails were removed every 12 hours (to reduce the chance of additional post-mortality shell damage) for the duration of the experiment (7 days). The experiment was repeated using wild snails (of comparable shell breadths) from populations with conspicuous differences in shell thickness (Wizard Islet; Grappler Inlet). This second predation trial allowed us to compare the adaptive value of shell thickness variation occurring only near the shell lip (characteristic of snails from the induction experiment) versus shell thickness variation existing throughout the shells (characteristic of snails from wild populations). Again, snails were individually marked for later identification. Logistic regression tested whether inter-individual differences in shell thickness related to survivorship after 7 days.

3. RESULTS

ANCOVA confirmed homogeneity of slopes (all interactions involving covariates were not significant; $p > 0.25$), allowing for direct tests of treatment effects. Somatic growth rate varied significantly among treatments ($F_{5,42} = 9.95$, $p < 0.0001$) beyond variation due to differences in initial body mass ($F_{1,42} = 15.58$, $p = 0.0003$). Snails exposed to the scent of large *Cancer* crabs (figure 1) grew the slowest. Final shell thickness varied with both shell breadth ($F_{1,35} = 11.35$, $p = 0.002$) and somatic growth rate ($F_{1,35} = 5.77$, $p = 0.022$), although these covariates were correlated ($F_{1,35} = 3.88$, $p = 0.057$), making their individual effects difficult to distinguish. Nonetheless, shell thickness intercepts varied among treatments ($F_{5,35} = 6.16$, $p = 0.0003$). Shells exposed to *Cancer* effluent grew thicker than those exposed to *Carcinus* effluent (figures 1 and 2).

When experimental *Nucella* were offered to *Carcinus*, there was no relationship between shell lip thickness and survivorship ($\chi^2 = 0.19$, $p = 0.66$, $n = 72$), unlike for wild snails where the likelihood of survivorship increased significantly with shell thickness ($\chi^2 = 11.56$, $p = 0.0007$, $n = 72$) (figure 3).

4. DISCUSSION

Avoidance behaviour (e.g. hiding) increases the chance of evading a would-be predator at a cost of reduced time foraging and therefore reduced somatic growth. Such a cause of reduced growth is common in gastropods, and seems to occur collaterally with an added benefit (assuming a predator does indeed lurk): increased shell thickness (Palmer 1990; Trussell & Nicklin 2002; Brookes & Rochette 2007). This effect was apparent in *Nucella*, insomuch that whelks in the low-food treatment produced thicker shells than continuously fed controls (figure 1) and, though not quantified, whelks in the *Cancer* treatment appeared to have eaten fewer barnacles than those in controls. Moreover, even when final shell thickness was adjusted for differences in somatic growth, *Nucella* nonetheless developed thicker shells when exposed to *Cancer* than controls, suggesting this alteration of shell form was an adaptive response to the detection of a shell-crushing predator.

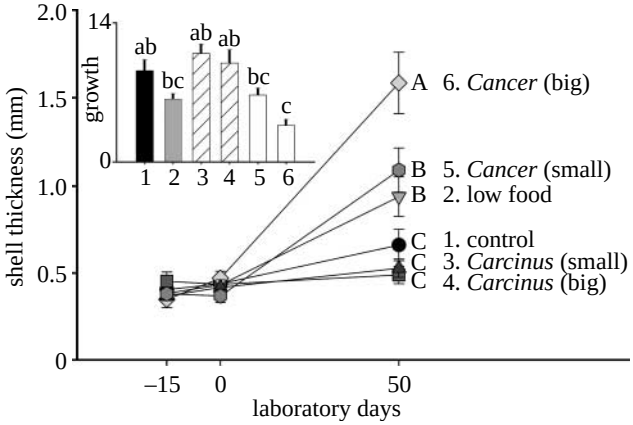

Figure 1. Shell thickness as a function of time in *N. lamellosa* snails under six experimental treatments: upon collection (−15 days), when treatments began (day 0) and at the end of the experiment (day 50). Inset: summary of somatic growth rate (mg d^{-1}) between days 0 and 50 of the experiment. Treatments included the native red rock crab *C. productus* and the exotic European green crab *C. maenas*. Common letters indicate similar mean effects (Tukey's $p < 0.05$). Standard error bars.

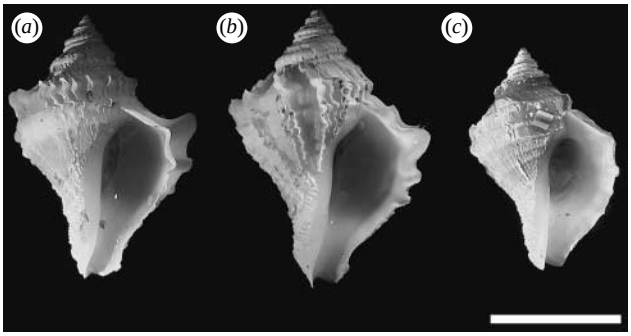

Figure 2. Final shell forms of *N. lamellosa* from three treatment groups (*a*) control, (*b*) *Carcinus* (big) and (*c*) *Cancer* (big). Shell sizes were similar at the beginning of experiment. Scale bar, 10 mm.

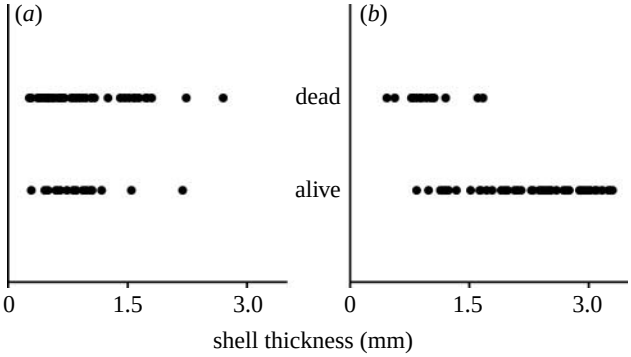

Figure 3. Summary of predation experiment showing survivorship of experimental snails as a function of shell lip thickness for (*a*) experimental ($p = 0.66$) and (*b*) wild snails ($p = 0.0007$).

We assume, of course, that thicker shells increase protection against shell-breaking predators (like *Cancer* and *Carcinus*). We were therefore surprised that the threefold increase in shell thickness induced by *Cancer* did not increase survivorship among whelks fed directly to *Carcinus*. Post-predation shell fragments revealed an unexpected explanation for this paradox:

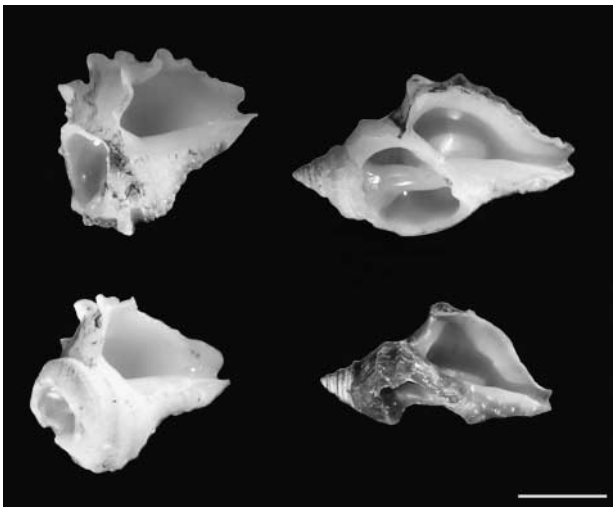

Figure 4. Induced thickening at the shell lip offers no fitness advantage when crab predators attack old 'thinner' shell whorls. Scale bar, 10 mm.

in nearly half of all predation events, *Carcinus* attacked older thinner shell whorls, leaving the newer thicker shell growth unscathed (figure 4). Therefore, we suspect that induced shell thickening may only increase fitness when the enhancement begins at a small enough shell size where older, more vulnerable whorls are overgrown. This supposition was confirmed in a subsequent predation experiment involving wild snails—whose shells presumably differed in thickness throughout and not merely at the apertural lip—where thicker shells were less likely to be broken than thinner shells (figure 3).

That *Nucella* responded to a known predator (*Cancer*) but not a recently introduced one (*Carcinus*) suggests that adaptive phenotypic plasticity (in response to *Carcinus*-specific cues) is latent. Such latent plasticity could explain why Atlantic blue mussels (*Mytilus edulis*) that are sympatric with another invasive predator, the Asian shore crab (*Hemigrapsus sanguineus*), can be induced by *Hemigrapsus* scent to increase shell thickness, but conspecific mussels living outside this invader's current range show no such response (despite responding adaptively to the scent of other crab predators; Freeman & Byers 2006, but see counter arguments by Rawson *et al.* 2007).

How cue recognition and flexible developmental pathways might become coupled is a fascinating problem. Given the well-studied capacity for associative learning in gastropods (Hermann *et al.* 2007), entire populations may learn to recognize and respond to novel threats quickly by associating the new predator-specific (and unrecognized) odour with the recognized scent of conspecific alarm cues, explaining how apparent shifts in phenotype can occur rapidly in large numbers of individuals following a sudden change in an environment (like a species introduction). Given the pervasiveness of inducible defences and the capacity for associative learning in animals, we suggest this may be a particularly relevant, and fruitful, direction for future research.

Protocols conformed to guidelines set by the BMSC Animal Care Committee.

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