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Retained non-adaptive plasticity: gene flow or small inherent costs of plasticity?

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ABSTRACT

Question: Do clams from populations not exposed to a predator retain the ability to respond to that predator?

Motivation: If maintaining the potential for phenotypic plasticity involves a significant cost, plasticity should be selected against in constant environments.

Background: Clams of the species *Macoma balthica* (a burrowing bivalve) respond to shore crabs by burrowing deeper in the sediment. Norwegian *M. balthica* are not exposed to crabs naturally, whereas Dutch *M. balthica* are naturally exposed to variable crab densities.

Sites: *Collection:* the Balsfjord near Tromsø, Norway, and the Wadden Sea near Harlingen, The Netherlands. *Holding tanks:* outdoor basins with a continuous flow of unfiltered water from the Wadden Sea.

Method: We introduced a mixture of clams from both sites into experimental aquaria with a thick layer of sandy sediment. Twelve aquaria contained one shore crab; twelve had none. We measured burrowing depth 7 days after the start of each experiment.

Result: Clams from the two sites show similar burrowing responses after exposure to predatory crabs, supporting the hypothesis that maintaining the potential for plasticity costs very little.

Keywords: anti-predation behaviour, bivalve, inherent cost, *Macoma*, phenotypic plasticity.

INTRODUCTION

Phenotypic plasticity is recognized as a potential evolutionary solution to the problems posed by living in a variable environment (Schlichting and Pigliucci, 1998; Piersma and Drent, 2003). Natural selection is thought to favour phenotypic plasticity over fixed phenotypes if: (1) there is spatial or temporal variability in a selective factor, (2) there is information available to the individual to predict the future environment reliably, (3) the alternative phenotypes increase fitness in the environment in which they are expressed, (4) this (heritable) increase in fitness is larger than the fitness costs of plasticity (Tollrian and Harvell, 1999).

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DeWitt *et al.* (1998) review the costs of plasticity [see Tollrian and Harvell (1999) for a somewhat different breakdown]. Some of these relate to the costs of *expressing* plasticity, and most empirical studies have focused on such expression costs (Tollrian and Harvell, 1999). However, there should also be costs to the *capacity* to express plasticity. We term these the 'inherent costs'. These may include costs for maintaining the genetic coding for plasticity (note that we do not discuss learned plasticity), costs for the sensory systems needed to acquire information on the environment, costs for developing and maintaining biochemical, neurological and motor systems that have to operate when called for, and energetic and ecological costs of wrongly expressed plasticity. Given any 'inherent' costs of plasticity, then, in a constant environment (where plasticity provides no benefits) plasticity should be selected against.

Although the inherent cost of plasticity may play an important role in the evolution of plasticity (DeWitt *et al.*, 1998; Tollrian and Harvell, 1999; Pigliucci, 2001), few attempts to measure it have been published (Nguyen *et al.*, 1989; Krebs and Feder, 1997; DeWitt, 1998; Scheiner and Berrigan, 1998; Relyea, 2002; Loman and Claesson, 2003; Steinger *et al.*, 2003). Most did not succeed, and some even suggest the existence of positive costs (i.e. benefits)! Scheiner (2002) suggests that further development of models assuming an inherent cost might be a waste of time.

The inherent cost of plasticity has been estimated by genotypic engineering to increase plasticity or by comparing fitness among families that differ in plasticity. The low success in measuring inherent plasticity costs suggests that we should look for alternative methods (Pigliucci, 2001). In this paper, we propose such an alternative method – that is, a comparison of the capacity to express plasticity between a population that no longer benefits from a particular kind of plasticity and an ancestral population in which plasticity remains adaptive. If the inherent cost to plasticity is large, selection should be quick to remove it from the derived population, but, if the inherent cost is small, plasticity may persist for a long time. As an example of this method, we present some of our own data and results.

MATERIALS AND METHODS

We exposed individuals of the bivalve *Macoma balthica* (L.) (Tellinidae) to shore crabs *Carcinus maenas* and measured their anti-predation behaviour [burrowing deeper into marine soft-sediments (Edelaar, 2000)]. At intertidal sites in The Netherlands, shore crabs are common, with on average 0.11 individuals per square metre (Adema, 1991). At intertidal sites in northern Norway, however, shore crabs are absent (Verwey, 1978; Vader, 1979) and other crabs do not occur. Norwegian coastal regions were unsuitable to *M. balthica* during the last glaciation (Luttikhuisen *et al.*, 2003), and a coastal current runs from south to north. We therefore infer that the Norwegian clam population without crabs is derived from a more southerly clam population with crabs, instead of the reverse. Given their current absence, crabs have probably been absent at the site for the several thousands of years that clams must have been able to live here after the ice retreated.

Norwegian *M. balthica* were collected at the Balsfjord near Tromsø (Sørkjosen, 69.13°N) in the summer of 1999. We stored them in outdoor basins with a continuous flow of unfiltered water from the Wadden Sea. Dutch *M. balthica* were collected in May 2001 in the Wadden Sea near Harlingen (Ballastplaat, 53.15°N) and were stored in the same way. Thus both populations had time to adjust to a new but common environment. This decreases carry-over effects of exposure to earlier environments that may confound experimental results.

On 29 June 2001, we individually marked all clams (see Edelaar, 2000) and placed them intermixed on top of a thick layer of sandy sediment in each of 24 isolated identical aquaria (see Edelaar, 2000). Each aquarium held five Dutch (total $n = 120$) and three or four Norwegian (total $n = 88$) individuals. Each aquarium also contained a plastic mesh cage with a closed bottom hanging submerged in the water but not touching the sediment. In twelve aquaria, the cage contained one shore crab (adult male, carapace width 40–50 mm) not fed in the 2 weeks prior to the experiment. (This excludes cues from consumed prey.) In the other twelve aquaria, the cages were empty (controls). *Macoma balthica* responds to shore crabs by burrowing deeper in the sediment (Edelaar, 2000). Previous experimental results (Edelaar, 2002) show that this response is most likely induced by water-borne info-chemicals (tactile and visual stimuli were experimentally excluded) specific to crabs.

We first measured burrowing depth 7 days after the start of the experiment. We then switched the individuals in a particular aquarium with individuals in an aquarium of the opposite treatment (keeping the groups together) and we allowed them to reburrow. This paired switching effectively equalizes treatment order. Two days later, we made a second measurement of burrowing depth [see Edelaar (2000) for high repeatability of burrowing depth].

For reasons of statistical independence, for each aquarium pair and for each source population separately, we used the average change in burrowing depth (crab treatment minus control treatment). We then calculated the ‘exposure effect’ for each source population as the average of this change for the two groups of clams using the same aquarium pair. For example, if in aquarium pair 1 a group of Dutch clams undergoing ‘increased risk’ first burrowed 10 mm in the control treatment and then 17 mm with a crab, and the other group undergoing ‘decreased risk’ first burrowed 15 mm with crabs and then 11 mm in the control treatment, the exposure effect would be $[(17 - 10) + (15 - 11)]/2 = 5.5$ mm.

Normal healthy clams burrow until they are completely covered (Edelaar *et al.*, 2003). Therefore, we excluded data of individuals that did not burrow or that died (8.7%). This resulted in the loss of one pair of aquaria, as one of the Norwegian groups (three clams) had no remaining data. We checked the distributions of the exposure effects for normality and homogeneity of variances, and no transformations were needed.

The exposure effect represents the average effect of exposure to shore crabs on burrowing depth for each of the two source populations in the same aquarium pair. If plasticity is indeed selected against, we predict that the exposure effect of the Dutch clams should exceed that of the Norwegian clams. So we calculated the difference in exposure effect between the two source populations (The Netherlands minus Norway) in each aquarium pair (i.e. a genotype \times environment interaction). With a one-sample *t*-test, we tested the null hypothesis that the mean of these differences in exposure effects was zero (equivalent to a paired *t*-test).

Based on previous results (Edelaar, 2000), we expected the changes in burrowing depth to be positive (deeper, not shallower, burrowing when a shore crab is present). We therefore used one-tailed tests. Similarly, since we predicted that Norwegian clams would respond less than Dutch clams to shore crabs, we also used a one-tailed test for that difference.

RESULTS AND DISCUSSION

Clams which moved from the control to the crab treatment burrowed deeper (Table 1). However, clams which moved from the crab to the control treatment also burrowed deeper,

Table 1. Change in burrowing depth (predator minus control measurement, in millimetres) of *Macoma balthica* from intertidal populations in The Netherlands and Norway (mean \pm standard error)

Source habitat	Increased risk	Decreased risk	Exposure effect	t_{10}	P
The Netherlands	17.4 \pm 2.44	-7.32 \pm 2.08	5.04 \pm 1.95	2.58	0.014
Norway	8.66 \pm 1.71	-2.77 \pm 1.83	2.95 \pm 1.44	2.05	0.034
Difference			2.10 \pm 1.56	1.34	0.11

Note: Clams were moved from control to shore crab treatment (increased risk), or from shore crab to control treatment (decreased risk). The exposure effect is the average effect of exposure to the predator on burrowing depth (average of second and third column). The third row gives the difference in exposure effect between the two source populations (first minus second row). For all exposure effects, the t -value and one-sided P -value are given.

producing negative values (Table 1). We believe that all clams were responding to the disturbance of our pulling them from the sediment to measure burrowing depth. In that case, all clams would perceive an increased risk in the environment between the first and second measurement. In any case, almost all our previous experiments using a similar design showed this disturbance effect. However, the exposure effect was positive (Table 1). So in addition to the equal disturbance effect, the change in crab presence did result in deeper burrowing.

The Norwegian clams responded to exposure to shore crabs in a way quantitatively similar to the Dutch clams (although a weak trend towards a decreased Norwegian response is visible in Table 1). This result contradicted our prediction that Norwegian clams would not respond to crabs. We discuss three potential explanations for the contradiction.

A lack of statistical power (Type II error)

We think that our statistical power is larger than the small sample size ($n = 11$) might suggest. First, the actual numbers used here are very strong estimates of a potential difference between source populations, because they are based on: (1) changes in burrowing depth between treatments (correcting for individual differences in values); (2) averages of changes in burrowing depth of several individuals within an aquarium (further averaging out individual differences in change of values); and (3) differences in averages of changes in burrowing depth between pairs of aquaria with opposite treatment (correcting for aquarium differences). Second, one-tailed tests provide extra statistical power, but still did not yield a significant difference between the two populations. Third, Norwegian clams responded significantly to shore crabs (Table 1). So they have not lost their response. Even if a larger sample size were to detect a decreased response in the Norwegian population (which is not at all certain), the enduring presence of the response would remain to be explained.

Gene flow

Selection may act to reduce unused plasticity (based on its inherent cost), but gene flow may prevent the complete disappearance of plasticity (cf. Storer and Sih, 1998). Indeed, there may be (or may have been) gene flow into the northern Norwegian population from southern

populations exposed to shore crabs. Gene flow would be the usual, acceptable and 'safe' explanation for our result.

However, genetic differentiation between Dutch and Norwegian populations is significant [based on mtDNA sequences (Luttikhuisen *et al.*, 2003)], suggesting some restrictions to gene flow. Moreover, inland fjords, such as the one where we collected our clams, are often relatively closed ecosystems with genetically differentiated populations of other species (W. Vader, personal communication), so gene flow may be highly restricted. Furthermore, predation on nearby non-fjord, oceanic populations of *M. balthica* by shore crabs is probably low or absent in northern Norway because crab densities there are very low. Also, low water temperature decreases the crab's crushing power, perhaps preventing even those few crabs from preying on clams (Vermeij, 1993; W. Vader, personal communication).

Small inherent costs of plasticity

If indeed gene flow is low enough (as we think it is) for selection to successfully reduce costly plasticity, the maintenance of the capacity to respond to shore crab exposure in the Norwegian population might be explained by inherent costs of plasticity that are so small that it takes a long time for selection to remove plasticity.

No published studies have specifically used our method of comparing populations to estimate the inherent cost of plasticity, although some studies have reported 'appropriate' levels of plasticity when comparing populations in different environments. However, in line with our results, a few other studies have documented the continued existence of behavioural plasticity that is currently unused in the natural situation (reviewed by Coss, 1999; Rydell *et al.*, 2000; Richardson, 2001). Some of these systems suffer even less from potential gene flow than in our study. Thus, together with our results, these findings suggest that plasticity may cost very little.

Very slow decay rates of plasticity in isolated, unexposed populations agree with the small or absent inherent cost of plasticity reported in the handful of studies that have measured inherent costs of plasticity in other ways (Nguyen *et al.*, 1989; Krebs and Feder, 1997; DeWitt, 1998; Scheiner and Berrigan, 1998; Relyea, 2002; Steinger *et al.*, 2003; Loman and Claesson, 2003). However, other studies have documented fast decay of anti-predation behaviours in isolated populations without predators. For example, Juliano and Gravel (2002) reported reduced expression of anti-predator behaviour in tree-hole mosquitoes after only two generations of no exposure to predators. Differences in the tendency of plasticity to persist might be explained in several ways. These include differences in inherent cost, but we have much more to learn before we can answer this question.

The disappearance of unused plasticity can be determined by focusing on presence/absence, but is better estimated in a quantitative way when possible (as in this study). Merilä and Crnokrak (2001) review a method for quantitative traits that can differentiate between decay by genetic drift (a null hypothesis for the disappearance of plasticity when not used anymore) and reduction by selection. This method is based on the notion that if degree and time of isolation of populations is not perfectly known, differentiation in neutral genetic markers (as given by F_{st} values) provides an estimate for differentiation in quantitative traits (as given by Q_{st} values) by drift. This elegant, recent method to correct population comparisons for shared ancestry and gene flow has almost exclusively been used for morphological and life-history traits. But it could also be applied to plastic (behavioural) quantitative traits (cf. Steinger *et al.*, 2002) if estimates of additive genetic variation

for plasticity can be obtained. If the decrease in plasticity is found to exceed decay by drift, further tools of quantitative genetics may be used to arrive at a quantitative estimate of the inherent cost of plasticity.

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