



## Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail

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### Abstract

Temporal and spatial patterns of phenotypic variation have traditionally been thought to reflect genetic differentiation produced by natural selection. Recently, however, there has been growing interest in how natural selection may shape the genetics of phenotypic plasticity to produce patterns of geographic variation and phenotypic evolution. Because the covariance between genetic and environmental influences can modulate the expression of phenotypic variation, a complete understanding of geographic variation requires determining whether these influences covary in the same (cogradient variation) or in opposing (countergradient variation) directions. We focus on marine snails from rocky intertidal shores as an ideal system to explore how genetic and plastic influences contribute to geographic and historical patterns of phenotypic variation. Phenotypic plasticity in response to predator cues, wave action, and water temperature appear to exert a strong influence on small and large-scale morphological variation in marine snails. In particular, plasticity in snail shell thickness: (i) may contribute to phenotypic evolution, (ii) appears to have evolved across small and large spatial scales, and (iii) may be driven by life history trade-offs tied to architectural constraints imposed by the shell. The plasticity exhibited by these snails represents an important adaptive strategy to the pronounced heterogeneity of the intertidal zone and undoubtedly has played a key role in their evolution.

### Introduction

Understanding the mechanisms that produce geographic variation is one of the basic pursuits of evolutionary studies (Endler, 1977). Although it has long been recognized that phenotypic variation reflects both genetic and environmental influences (Schmalhausen, 1949; Dobzhansky, 1951; Bradshaw, 1965), most early work considered geographic variation to be genetically based, adaptive and molded by natural selection (e.g., Mayr, 1963). Until recently, the importance of environmentally induced phenotypic plasticity was largely ignored both theoretically and empirically (Schlichting, 1986, 1989; Stearns, 1989; West-Eberhard, 1989). Over the past 15 years, a burgeoning body of evidence suggests that plasticity may underlie much geographic variation and has profound implica-

tions for understanding evolution (James, 1983; Schlichting, 1986; Stearns, 1989; West-Eberhard, 1989; Gotthard & Nylin, 1995; DeWitt, Sih & Wilson, 1998; Schlichting & Pigliucci, 1998).

Plasticity influences the evolution and adaptive responses of organisms because it can alter the relationship between the phenotype, which is the target of selection, and the genotype. The response of a trait to selection depends on its heritability and its genetic correlation to other traits also under selection (Roff, 1997). Plasticity can affect both of these factors and thus produce quite surprising effects on the direction and rate of evolution. For example, plasticity can transform a positive genetic correlation between two traits in one environment, to a negative correlation in another environment (Stearns, DeJong & Newman, 1991; Stearns, 1992). As a result, in the first envir-

onment selection to increase trait 1 would bring about an increase in trait 2, but in the second environment, would produce a decrease in trait 2.

Environmentally induced phenotypic variation can have several other important effects, most easily understood by considering reaction norms, the set of phenotypes produced by a single genotype across an environmental gradient. When reaction norms cross, as they often do (Stearns, 1992), it will modulate heritability in different environments. If all reaction norms cross in one environment, phenotypic variation and heritability will be zero, and genotypes can not be distinguished by natural selection. Away from the crossing point, heritabilities will be nonzero and genotypes will differ phenotypically. Crossing reaction norms can also change the rank order of phenotypes (Via & Lande, 1985) such that the genotype that is favored may vary among environments, even if the selection pressures are constant (e.g., for large body size). In this way, plasticity provides one mechanism for maintaining genetic variation (Gillespie & Turelli, 1989) because it reduces the probability that a single genotype will be optimal in all environments. Plasticity can therefore influence which genotypes are favored in particular environments, how populations respond to selection, the rate at which genotypes are fixed and the maintenance of genetic variation.

Plasticity itself may be under genetic control and evolve, although the nature of the mechanisms are controversial. The debate centers on whether plasticity simply reflects selection for different trait means in different environments (Via & Lande, 1987; Via, 1993; Via et al., 1995), or whether there exist specific loci that control the form and degree of plasticity (Scheiner, 1993a, b; Schlichting & Pigliucci, 1993). Two types of loci have been identified, regulatory loci that alter gene expression across environments and loci with allelic sensitivity where different alleles are expressed in different environments. The extent to which these shape plasticity is not well understood. Reaction norms may evolve in response to both (Via et al., 1995), but the relative importance of each will vary with the nature and scale of environmental heterogeneity and the type of trait involved. It is critical to identify the role these mechanisms play because they will ultimately determine the rates and constraints on phenotypic evolution.

Maintaining an environmentally sensitive phenotype involves a variety of costs and limits which can constrain the evolution of plasticity as well as the traits involved (DeWitt, Sih & Wilson, 1998). A cost

occurs when a plastic organism produces the same mean phenotype as a fixed organism in a particular focal environment, but attains a lower fitness. The lower fitness may occur because the plastic organism expends more energy producing the mean phenotype reducing energy available for reproduction. A limit occurs when plastic development can not produce as near an optimum phenotype as a fixed pathway in a particular focal environment.

Identifying the relative importance of genetic and environmental forces in shaping geographic variation therefore, is essential to understanding adaptation and evolution. Unfortunately, this is often nontrivial. The ideal procedure is to rear clones in different environments to estimate the components of phenotypic variation due to genetic makeup, environmental sensitivity and genotype  $\times$  environment interactions. For most organisms though, clones are not available and one needs to rely on closely related groups of individuals (full-sibs, half-sibs, demes, etc). Under these circumstances, estimating the various components of phenotypic variation is considerably more complicated and fraught with a variety of problems (Stearns, 1992; Schlichting & Pigliucci, 1998). For example, if  $G \times E$  interactions are significant, one can not easily partition variation into genetic and environmental components or identify additive genetic effects. Nevertheless, a considerable amount of empirical work has quantified the contribution of genetic and environmental factors in producing patterns of geographic variation in a wide variety of organisms and habitats (Schlichting & Pigliucci, 1998). Our goal is to consider what has been learned about plasticity in shaping phenotypic variation and the evolution of intertidal organisms.

The rocky intertidal zone is one of the most variable environments on earth (reviewed in Denny, 1988; Bertness, 1999) and thus is a convenient setting for testing theories about evolution in heterogeneous environments. Much of this variation can be attributed to temporal and spatial variation in wave action that strongly influences the biotic and abiotic conditions under which organisms must live (e.g., Dayton, 1971; Palumbi, 1984; Denny, Daniel & Koehl, 1985; Wethey, 1985; Etter, 1989; Trussell, 1996, 1997a, b). Breaking waves can impart forces on intertidal organisms ranging from a slight trickle to a raging torrent where water velocities reach 20 m/s with accelerations of  $500 \text{ m/s}^2$  (Denny, Daniel & Koehl, 1985). Drag components of such velocities would be tantamount to wind velocities in excess of 460

m/s (1000 mph) in an analogous terrestrial situation (Vogel, 1981).

It is not surprising therefore, that many intertidal organisms exhibit morphological variation that parallels gradients in wave exposure (Crothers, 1983; Janson, 1983; Etter, 1988a, b; Dudgeon & Johnson, 1992; Trussell, 1996, 1997a, b). For example, sponges produce stiffer tissues with smaller conducting elements under high wave energies relative to low (Palumbi, 1984, 1986) and shallow water corals often have branching morphologies that depend on exposure regime (Chamberlain & Graus, 1975; Graus, Chamberlain & Boker, 1977). The exact mechanisms producing this variation and to what extent the variation represents genetic differentiation or environmental responses is not well understood for most organisms. The gastropods have been the most thoroughly studied and we focus on them. The results indicate that spatial and temporal patterns of morphological variation reflect complex genetic and plastic responses to several interdependent environmental factors.

### Small-scale patterns of geographic variation

#### *Wave action*

Marine snails on temperate rocky intertidal shores exhibit substantial morphological variation that is often correlated with dramatic environmental gradients, even on very small spatial scales (tens of meters). Most studies on habitat-specific patterns of morphological variation have focused on wave-exposed and sheltered populations (Kitching, Muntz & Ebling, 1966; Johannesson, 1986; Etter, 1988a; Boulding, 1990; Boulding & Van Alstyne, 1993; Trussell et al., 1993; Trussell, 1996, 1997a), a fruitful dichotomy because of the distinctive selection regimes.

On wave-exposed shores, hydrodynamic constraints imposed by breaking waves are thought to be the primary selective force acting on snail morphology (Denny, Daniel & Koehl, 1985; Denny, 1988; Trussell, 1997a, b). Increased turbulence and wave splash is thought to diminish the impact of crab predation on wave-exposed shores as well as reduce desiccation stress in middle and lower sections of the shore during low tide. Snails in these environments typically have a large adhesive foot that improves their ability to remain attached to the substratum (Kitching, Muntz & Ebling, 1966; Etter, 1988a; Trussell, 1997a). Moreover, the smaller-sized and squatter shells of

wave-exposed snails may reduce their risk of dislodgement by: (1) reducing the amount of drag on their shell (Trussell et al., 1993; Trussell, 1997a) and (2) increasing their ability to exploit microhabitats, thus reducing their exposure to free-stream flows (Denny, Daniel & Koehl, 1985; Trussell, 1997b).

#### *Predation*

On sheltered shores the impact of wave action is negligible and crab predation is thought to be the primary agent of selection (Kitching, Muntz & Ebling, 1966; Palmer, 1985; Johannesson, 1986). Desiccation stress also can be important in these habitats, particularly in the absence of thermal buffering afforded by a dense macroalgal canopy (e.g., *Ascophyllum nodosum*). Patterns of morphological variation in sheltered snails are consistent with predictions for the effects of crab predation and desiccation stress. Snails on these shores are typically lighter in color (Etter, 1988b) and larger and thicker (Kitching, Muntz & Ebling, 1966; Reimchen, 1982; Johannesson, 1986; Trussell, 1996). Lighter shells can reduce the magnitude of solar gain (Etter, 1988b) while thicker shells are more difficult for predatory crabs to crush (Kitching, Muntz & Ebling, 1966; Bertness & Cunningham, 1981; Palmer, 1985; Seeley, 1986).

### Large scale patterns of geographic variation

#### *Predation*

The importance of shell crushing predators (decapod crustaceans, fish) to the evolution of gastropod shell form also is suggested by large-scale studies (Vermeij, 1976, 1978, 1982, 1987; Palmer, 1979; West & Cohen, 1996). For example, paleontological data indicate that post-Paleozoic fossil shells show higher frequencies of shell repair (Vermeij, Schindel & Zisper, 1981) and more robust, better defended shell morphologies (e.g., lower spires, thicker shell walls and apertural lips, narrow apertures) than Paleozoic assemblages (Vermeij, 1987). These morphological shifts coincided with the diversification of shell crushing predators in the Mesozoic (Vermeij, 1977).

Biogeographic evidence suggests that gastropod species have more robust shell morphologies in regions where shell crushing predators are more taxonomically diverse and powerful and where there has been a longer time for co-evolution between predator and prey (Vermeij, 1978, 1987; Vermeij & Veil, 1978).

For example, tropical gastropod shells are more robust than temperate snails (Vermeij, 1978; Vermeij & Currey, 1980); Indo-West Pacific snails are better defended than Caribbean congeners (Vermeij, 1976); and freshwater snails from ancient African rift valley lakes are stronger than snails from nearby, but younger lakes (West, Cohen & Baron, 1991).

Perhaps the most compelling evidence supporting the role of crab predation as a force driving the evolution of gastropod shell form comes from the comparative studies of Vermeij (1982) and Seeley (1986). They documented 'rapid' historical transitions in the shell form of two intertidal species (*Nucella lapillus* and *Littorina obtusata*) that coincided with the geographical range expansion of the invasive green crab (*Carcinus maenas*) into the Gulf of Maine (GOM). The green crab was introduced to the mid-Atlantic coast of the United States from its native Europe in the 1800's, but the northern border of its range in North America until 1900 was Cape Cod, Massachusetts. Around 1900, this crab began to colonize intertidal shores north of Cape Cod, reaching Portland, Maine in the early 1900's, mid-coastal Maine by the 1930's, and northeast Maine and the Canadian Maritimes by the 1950's (Scattergood, 1952; Welch, 1968). Presently, this crab is distributed throughout the GOM, but populations in northern Maine and the Bay of Fundy are often small and extremely patchy (Seeley, 1985, 1986).

By comparing museum specimens collected from New England localities before and after the green crab invasion, Vermeij (1982) and Seeley (1986) found that post-invasion shells of both *Nucella lapillus* and *Littorina obtusata* were thicker. Seeley's (1986) work in particular has been cited (Gingerich, 1993; Thompson, 1998; Hendry & Kinnison, 1999) as an example of rapid evolution with green crab predation acting as the driving force.

#### *Water temperature*

The environmental effects of water temperature can influence multiple properties of calcium carbonate based shells. Because  $\text{CaCO}_3$  availability decreases and  $\text{CaCO}_3$  solubility increases with decreasing water temperature, both the deposition and maintenance of shells are expected to be more difficult in colder waters (Graus, 1974; Vermeij, 1978, 1993). This prediction is supported by increased calcification indices (the ratio of shell mass to its internal volume) in tropical versus temperate molluscs (Graus, 1974; but see

Vermeij, 1993). Moreover, both Lowenstam (1954a, b) and Dodd (1963) found that calcite:aragonite ratios in *Mytilus edulis* increased with latitudinal decreases in water temperature. This latitudinal trend in calcite:aragonite ratio likely reflects the relatively higher solubility of aragonite in colder waters (Pytkowicz, 1969). In terms of shell strength, these mineralogical differences may have functional consequences because, compared to aragonite, calcite is softer, less dense, and tends to break along well-defined cleavage planes (Carter, 1980). Thus, for gastropods distributed along a latitudinal temperature gradient, shells in colder waters are likely to be thinner, microstructurally weaker, and more vulnerable to crushing predators than those in warmer waters.

The influence of water temperature on shell form also appears to operate on regional scales. For example, water temperatures in the GOM during the spring and summer, when most snail growth occurs, average  $\sim 6^\circ\text{C}$  colder in northern (Maine-Canadian border) versus southern (Massachusetts) localities (Trussell, 2000a). Snails (*Littorina obtusata*) from the northern GOM are significantly thinner than those from the southern GOM and this pattern appears to partly reflect plasticity in response to geographic differences in water temperature (Trussell, 2000a; Trussell & Smith, 2000). Thus, it is likely that latitudinal differences in water temperature contribute to biogeographic variation in gastropod shell form.

Clearly, both differences in shell crushing predators and water temperature can contribute to global and regional differences in gastropod shell form. Unfortunately, quantifying the relative importance of each is difficult. Recent experiments (see below) have attempted to address how green crab predation and water temperature interact on a regional scale to shape latitudinal differences in shell thickness (Trussell, 2000a; Trussell & Smith, 2000).

#### **Phenotypic differentiation: the role of genetic differentiation and plasticity**

An understanding of genetic versus plastic contributions to phenotypic variation has direct bearing on the reliability of our interpretations of phenotypic change in ecological and geological time (i.e., the fossil record). For example, Williamson (1981) documented morphological changes in several sexual and asexual Cenozoic molluscan lineages from the Turkana Basin that seemed consistent with the punctuated equilib-

rium view of evolution. Although Williamson's interpretation may be correct, several subsequent commentaries argued that such transitions also could reflect ecophenotypic changes in response to environmental changes associated with rising and falling lake levels (Boucot, 1982; Charlesworth & Lande, 1982; Mayr, 1982).

In rocky intertidal systems, geographic variation and evolutionary shifts in molluscan shell form have traditionally been thought to largely reflect genetic differentiation shaped by natural selection via wave action and crab predation (Kitching, Muntz & Ebling, 1966; Vermeij, 1982, 1987; Johansson, 1986; Seeley, 1986; Boulding, 1990; Trussell, 1997a, b). Of course other factors are involved, but these two forces are generally thought to be the most important. Recent studies, however, suggest that natural selection has favored the evolution of phenotypic plasticity in response to wave action, predator cues, and water temperature and that such plasticity can influence small and large scale patterns of phenotypic variation (Appleton & Palmer, 1988; Etter, 1988a; Trussell, 1996, 1997a, 2000a,b; Palmer, 1990; Trussell & Smith, 2000). Phenotypic plasticity in response to these factors represents an important, and often underappreciated, explanation of spatial and temporal changes in molluscan shell form.

#### *Plasticity in foot size in response to increased wave action*

Although several studies have documented increased gastropod foot size on wave-exposed versus sheltered shores, to our knowledge only two studies have examined the basis of this pattern in detail. Both *Nucella lapillus* (Etter, 1988a) and *Littorina obtusata* (Trussell, 1997a) exhibit plasticity in foot size. Snails from sheltered populations raised in high and low flow environments in both the laboratory and field produced a larger adhesive foot in the high flow environments. Interestingly, both studies documented an asymmetry in foot size plasticity; snails from wave-exposed sites showed no plasticity in foot size between high and low flow environments. Natural selection may favor such an asymmetry if the cost of miscuing to prolonged calm periods on wave-exposed shores is greater than the benefits of plasticity (see Palumbi, 1984; Etter, 1988a; Trussell, 1997a). This asymmetry suggests two things. First, genetic differentiation in foot size plasticity may exist on relatively small spatial scales. Second, the reaction norms for foot size of

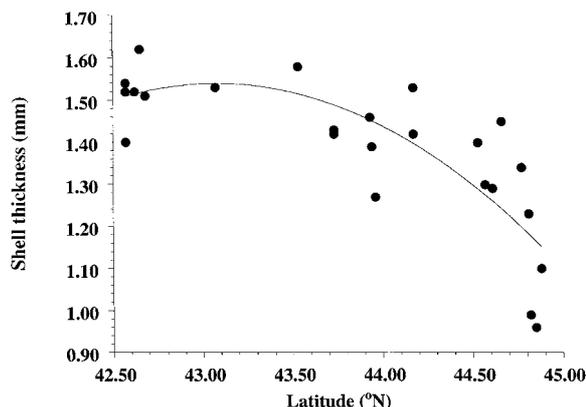


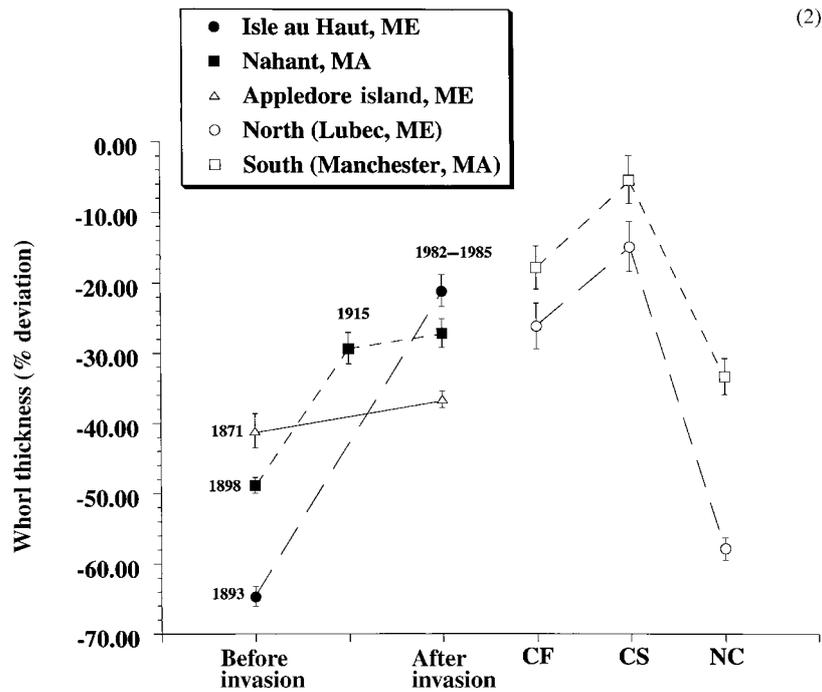
Figure 1. Shell thickness ( $Y$ ) as a function of latitude ( $X$ ) for 25 *Littorina obtusata* populations in the Gulf of Maine. Shell thickness decreases significantly with increasing latitude ( $Y = 10.28X - 0.12X^2 - 219.92$ ;  $R^2 = 0.65$ ;  $p < 0.0001$ ). Shell thickness of each snail was expressed as a deviation from a regression of log shell thickness ( $Y$ ) versus log shell length ( $X$ ) across all populations. Mean shell thickness was back-transformed for presentation and is expressed as a percent deviation from the common regression (see Trussell & Smith, 2000).  $N = 50$  for each population. Error bars are smaller than symbols.

wave-exposed snails may have evolved less flexibility compared to sheltered snails, perhaps so much so that foot size in wave-exposed snails is not plastic.

#### *Plasticity in shell thickness in response to risk cues from predators and conspecifics*

Geographic and paleontological studies support the idea of a strong coevolutionary relationship between shell crushing predators and their molluscan prey. Recent work suggests that phenotypic plasticity also may contribute to predator-prey coevolution (Smith & Palmer, 1994; Trussell & Smith, 2000). Inducible defenses are a form of phenotypic plasticity describing the production of morphologies, chemicals, or behaviors by prey in response to cues emitted by predators (Tollrian & Harvell, 1999). The study of inducible defenses has a long history in both freshwater zooplankton (Gilbert, 1966; Dodson, 1989) and terrestrial plant systems (Rhoades, 1979; Baldwin & Schultz, 1983; Karban & Baldwin, 1997; Karban, Agrawal & Mangel, 1997), but this phenomenon has only recently been considered in marine snail-decapod crustacean systems.

Despite a short history, it is increasingly clear that inducible defenses in marine molluscs are taxonomically and geographically widespread. Inducible increases in shell thickness in response to crab predator cues are known for several molluscan species



Figures 2–4. Comparison of historical shifts in three measures of shell thickness (Figure 2 = apertural lip thickness adjacent to the whorl; Figure 3 = apertural lip thickness opposite the whorl, Figure 4 = mean shell thickness, calculated as the mean of whorl thickness and opposite whorl thickness (see Trussell, 1996, 2000a, b; Trussell & Smith, 2000) for several *Littorina obtusata* populations before and after the invasion of the green crab (*Carcinus maenas*) into the GOM). Also shown are the same measures of shell thickness for northern and southern GOM *Littorina obtusata* that were experimentally raised in the laboratory under three risk cue treatments: NC = no risk cue (experimental control); CF = green crabs fed fish; and CS = green crabs fed conspecific snails. Both museum and laboratory specimens were measured by G.C. Trussell following Trussell (1996, 2000a, b) and Trussell and Smith (2000). Measures of shell thickness were back-transformed for presentation and are expressed as a percent deviation from the common regression (see Trussell & Smith, 2000). Museum lot numbers, sample sizes and the collection location of specimens are given in Table 1. Details of statistical analyses and percent deviations in shell thickness among different experimental groups are given in Table 2.

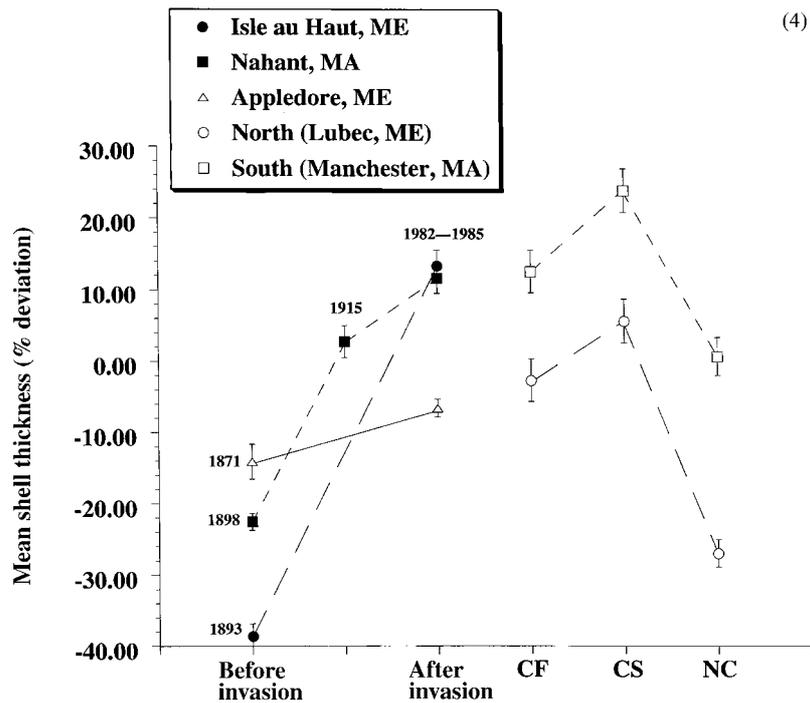
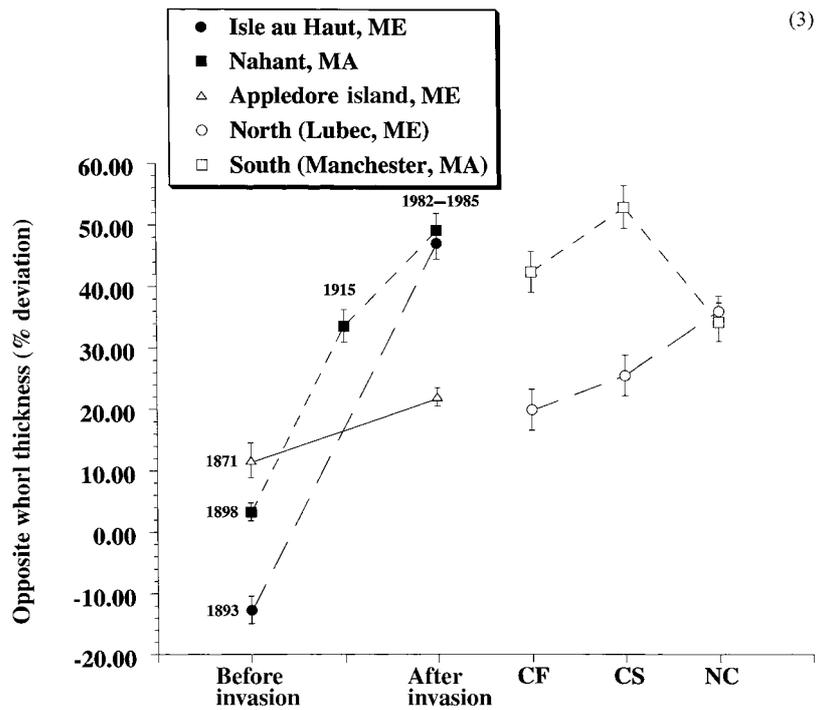
including *Nucella lapillus* from the northeast Atlantic (Palmer, 1990), *Nucella lamellosa* from the northeast Pacific (Appleton & Palmer, 1988), and *Mytilus edulis* (Leonard et al., 1999), *Littorina obtusata* (Trussell, 1996; 2000b; Trussell & Nicklin, in press), *Littorina littorea* (Trussell et al., unpublished), and *Nucella lapillus* (Nicklin & Trussell, unpublished) from the GOM. Such diversity (two genera of crab and three of mollusc) in the capacity of prey species to exhibit this plasticity, as well as the multiple predator species that induce it, suggests that predator-induced plasticity may be important in producing small and large-scale patterns of phenotypic variation.

#### What have we learned from the green crab invasion?

Because the invasion of the GOM by green crabs (*Carcinus maenas*) alluded to earlier is recent and spans a rather wide latitudinal range, it provides an excellent

opportunity to consider how historical and latitudinal differences in selection intensity may shape temporal and geographic patterns of morphological variation. Latitudinal variation in *Littorina obtusata* shell thickness in the GOM is consistent with that predicted by differences in historical contact with the green crab. Snails from the southern GOM, which have been exposed to green crab predation for at least 100 years, have significantly thicker and stronger shells than those from the northern GOM (Trussell, 2000a, b). Overall, shell thickness decreases by ~34% over a 400 km gradient from northern Massachusetts to the Maine-Canadian border (Figure 1).

Despite the intuitive appeal of rapid microevolution via directional selection imposed by green crab predation as an hypothesis explaining historical (*sensu* Vermeij, 1982; Seeley, 1986) and geographic phenotypic change, recent work suggests that phenotypic plasticity in response to green crab cues also may



Figures 2-4. Continued.

Table 1. Summary of *Littorina obtusata* museum specimens collected before and after the invasion of the green crab (*Carcinus maenas*) into the Gulf of Maine (GOM) and *Littorina obtusata* from a northern and southern site in the GOM that were raised in the laboratory under different risk cues

Location/group	Latitude–longitude	Date	Museum lot#	N	Mean shell length (±SE)
Museum and Field-collected specimens					
Appledore island, Maine	42° 57.0' N, 70° 35' W	1871	YPM-19351	30	8.71 (0.24)
		1982	YPM-19349	33	7.69 (0.17)
		1984	YPM-19352	18	
		1984	YPM-19353	11	
		1984	YPM-19354	9	
		1985	YPM-19350	66	
Isle au Haut, Maine	44° 04.3' N, 68° 38.3' W	1893	MCZ-013972	30	8.66 (0.23)
		1982	YPM-19356	19	8.74 (0.39)
		1984	YPM-19357	16	
		1984	YPM-19358	13	
		1985	YPM-19355	15	
Nahant, Massachusetts	42° 25.5' N, 70° 55' W	1898	MCZ-2000	100	6.95 (0.07)
		1915	YPM-19079B	20	9.77 (0.31)
		1915	YPM-19079C	30	
		1982	YPM-19345	15	8.23 (0.31)
		1982	YPM-19346	15	
		1985	YPM-19347	21	
		1985	YPM-19348	13	
Laboratory plasticity experiment					
Lubec, Maine	44° 49.21' N, 66° 57.97' W	1999			
Crab-fish				42	8.67 (0.12)
Crab-Snail				42	8.04 (0.11)
No-Crab				42	8.78 (0.13)
Manchester, Massachusetts	42° 33.79' N, 70° 46.19' W	1999			
Crab-Fish				48	8.48 (0.12)
Crab-Snail				48	7.94 (0.11)
No-Crab				48	8.52 (0.15)

Note that for post-invasion samples, multiple sites were sampled in a given geographic region and this sampling occurred in different years in the mid-1980's. However, for all historical comparisons, all sites within a region were pooled together before statistical analyses. Thus, the mean shell length (±SE) given for post-invasion samples is based on all samples. Mean shell lengths are given to illustrate that historical and plastic changes in shell thickness are unlikely to be a simple by-product of samples having different size ranges. Nevertheless, residual analyses were conducted to adjust for the potential effects of shell size on shell thickness. Heavily damaged specimens were not measured. N = sample size, MCZ = Museum of Comparative Zoology (Harvard University), YPM = Yale Peabody Museum (Yale University).

contribute to these morphological differences. Field and laboratory experiments utilizing *Littorina obtusata* from northern Maine and Massachusetts revealed that the presence of green crab cues can induce 10–47% increases in mean shell thickness in just 45–90 days (Trussell & Smith, 2000; Trussell & Nicklin, in press). The magnitude of these responses is especially impressive when compared to morphological changes documented by historical studies: Vermeij (1982) documented approximately a 12% increase in the shell thickness of Atlantic *Nucella lapillus* in 25–100 years and Seeley (1986) documented 50–80% increases (as

estimated from Figure 2 in her paper) in *Littorina obtusata* shell thickness over a period of ~85–112 years.

To examine this issue further, we obtained the museum specimens that were used to document historical changes in *Littorina obtusata* shell thickness before and after the green crab invasion (see Table 1 and Seeley, 1986, for details of specimens used). Seeley (1986) reported thickness data for the apertural lip opposite the shell whorl while Trussell (1996, 2000a, b) and Trussell and Smith (2000) reported mean thickness data that were yielded by measurements of the

Table 2. Percent deviations in shell thickness measurements for museum specimens of *Littorina obtusata* collected before and after the green crab (*Carcinus maenas*) invasion into the Gulf of Maine (GOM)

Location	Comparison	Percent deviation		
		WT%	OWT%	MT%
Museum specimens				
Isle au Haut, Maine	1893 v.s. 1980's	43.6 (<0.0001)	59.8 (<0.0001)	51.9 (<0.0001)
Appledore island, Maine	1871 v.s. 1980's	4.6 (0.0951)	10.3 (0.0019)	7.5 (0.0080)
Nahant, Massachusetts	1898 v.s. 1915	21.8 (<0.0001)	46.0 (<0.0001)	34.1 (<0.0001)
Nahant, Massachusetts	1915 v.s. 1980's	2.2 (0.4661)	15.6 (<0.0001)	8.9 (0.0050)
Laboratory experiment				
Lubec, Maine				
	CS v.s. CF	11.4 (0.0184)	5.6 (0.2338)	8.4 (.0497)
	CF v.s. NC	31.6 (<0.0001)	16.4 (<0.0001)	24.1 (<0.0001)
	CS v.s. NC	43.0 (<0.0001)	22.0 (<0.0001)	32.5 (<0.0001)
Manchester, Massachusetts				
	CS v.s. CF	12.4 (0.0072)	10.5 (0.0295)	11.3 (0.0081)
	CF v.s. NC	15.6 (<0.0001)	8.2 (0.0757)	12.0 (0.0023)
	CS v.s. NC	27.9 (<0.0001)	18.7 (<0.0001)	23.3 (<0.0001)

WT = apertural lip thickness adjacent to the whorl, OWT = apertural lip thickness opposite the whorl, MT = mean shell thickness, calculated as the mean of whorl thickness and opposite whorl thickness (see Trussell, 1996, 2000a, b; Trussell & Smith, 2000). Also shown are percent deviations in shell thickness measurements of northern and southern GOM *Littorina obtusata* that were experimentally raised in the laboratory under three risk cue treatments: NC = No risk cue (experimental control); CF = green crabs fed fish; and CS = green crabs fed conspecific snails. ANOVA on deviations produced from a common regression of each measure of log shell thickness ( $Y$ ) as a function of log shell length ( $X$ ) yielded significant among group differences for each measure of thickness: WT ( $F_{12,662} = 61.73$ ,  $p < 0.0001$ ), OWT ( $F_{12,662} = 64.91$ ,  $p < 0.0001$ ), MT ( $F_{12,662} = 71.34$ ,  $p < 0.0001$ ). Values in parentheses are  $p$  values generated by linear contrasts testing for significant differences among different groups.

apertural lip adjacent to and opposite the shell whorl. Thus, previous comparisons of historical changes in *Littorina obtusata* shell thickness and that produced by plasticity have been indirect.

We measured shell length, lip thickness adjacent to and opposite the whorl (see Trussell, 1996 for diagram) for all of the snails in all of the lots reported by Seeley (1986). For this reason, most of our sample sizes are larger than those reported in Seeley (1986). These independent measurements were used to compare the magnitude of historical change in three estimates of shell thickness (whorl thickness, opposite whorl thickness, mean thickness) to that obtained in laboratory experiments examining plasticity in shell thickness in response to green crab and conspecific alarm cues (Trussell & Nicklin, in press). In general, our analysis of historical data (Table 2; Figures 2–4) indicate that whorl thickness increased 5–44%, opposite whorl thickness increased 10–60%, and mean shell thickness increased 7–52% (see Table 2 for more details) in conjunction with the green crab invasion. These changes occurred over a period of ~85–112

years depending on the locale examined. Our data also indicate that the amount of historical thickening depended on the region from which samples were obtained. Snails from Isle au Haut, Maine showed the greatest change in shell thickness whereas those from Appledore island, Maine showed the least change (Table 2; Figures 2–4).

In addition, we found that plastic increases in shell thickness in response to risk cues in the laboratory were comparable to the amount of historical change (Table 2; Figures 2–4). Snails from the southern GOM raised with risk cues showed an 8–27% increase in shell thickness relative to non-risk controls, whereas northern snails raised with risk cues showed a 16–43% increase in shell thickness relative to non-risk controls. These responses occurred in approximately 120 days. Thus, it appears that the magnitude of predator-induced plasticity in *Littorina obtusata* shell thickness is comparable to changes previously attributed to rapid microevolution via natural selection. We are not suggesting that these processes are mutually exclusive. Indeed, natural selection is likely shaping both genetic

differentiation in shell thickness and shell thickness plasticity (Trussell, 2000a; Trussell & Smith, 2000) to produce geographic and historical variation in shell thickness.

The elegant work of Losos and colleagues on Bahamian Anoles also supports the hypothesis that plasticity may be an important component of evolutionary change (Schmalhausen, 1949; West-Eberhard, 1989). Losos, Warheit and Schoener (1997) documented adaptive shifts in lizard limb length on islands that were experimentally colonized with founder populations. In general, those islands having vegetation with broad perches had lizards with long limbs, whereas those having vegetation with narrow perches had lizards with short limbs. These changes in limb length were quite rapid (20 years). Recent work (Losos et al., 2000) demonstrated that plastic changes in limb length can be induced by altering the size of perches (narrow v.s. broad) available to lizards during ontogeny. Clearly more work is needed to determine the relative importance of genetic differentiation and plasticity to the adaptive differentiation documented by Losos and colleagues (see Losos et al., 2001). Such empirical approaches are crucial to evaluating the evolutionary significance of plasticity.

If natural selection on shell thickness plasticity is an important component of phenotypic change, one would expect the evolution of different reaction norms to occur in the GOM due to the latitudinal differences in the historical intensity of green crab predation. Theory predicts that increased plasticity should evolve in situations where environmental conditions are more variable (Van Tienderen, 1991; Tollrian & Harvell, 1999). In the case of crab predation, early contact was probably sporadic favoring plasticity. Because of the shorter contact history between the green crab and *Littorina obtusata* and the more patchy distribution of the green crab in the northern GOM (Seeley, 1985, 1986; Trussell, pers. obs.), one would expect increased plasticity to evolve in northern snails. In contrast, because of more constant predation pressure, southern snails should evolve less plasticity perhaps to the point that variation in shell thickness becomes more constitutively determined.

Experiments suggest that northern snails are more plastic than southern snails, though geographic differentiation in shell thickness plasticity is cue dependent (Trussell, 2000b; Trussell & Nicklin, in press). For example, northern snails exhibit nearly twice the shell thickness plasticity in response to green crab cues

compared to southern snails (Table 2; Figures 2–4). However, northern and southern snails exhibit very similar degrees of shell thickness plasticity in response to conspecific alarm cues (compare Table 2; Figures 2–4). These cues are likely released into the environment by conspecific snails when predators such as green crabs are present (Hadlock, 1980; Appleton & Palmer, 1988). Our results suggest that conspecific alarm cues may be used in response to other predators besides the green crab, thus decreasing the likelihood of geographic differentiation in snail sensitivity to them.

Historical variability in the impact of green crab predation across latitude appears to have favored the evolution of more flexible reaction norms in the northern GOM and less flexible reaction norms in the southern GOM. In addition, geographic differences in reaction norm intercept (Trussell, 2000b; Trussell & Nicklin, in press) suggest that these populations have diverged genetically. Even at juvenile stages, southern *Littorina obtusata* are considerably thicker than similar sized snails from the northern GOM, and southern snails consistently produce thicker shells than northern snails under particular risk treatments. Thus, the greater shell thickness of southern juveniles may reflect differences in reaction norm intercept or perhaps pre-collection differences in plasticity in response to geographic differences in crab cue concentrations. Nevertheless, geographic differences in reaction norm slope suggest that selection has favored the evolution of more strict genetic control of shell thickness in southern snails, potentially limiting their degree of plasticity in response to environmental cues.

#### *The role of plasticity induced by latitudinal differences in water temperature*

Geographic differences in water temperature within the GOM also appear to operate on *Littorina obtusata* shell form, thus complicating interpretations of the relative importance of predator-induced versus temperature induced differences. As mentioned earlier, water temperatures during the growing season can be ~6°C colder in the northern versus southern GOM. The environmental effects of colder waters in the northern GOM are expected to lead to the production of thinner shells and reciprocal transplant experiments support this prediction (Trussell, 2000a). Northern snails transplanted to warmer southern waters produced shells 43% thicker than northern snails raised at their native site, whereas southern snails transplanted to a northern site produced shells 18% thinner than

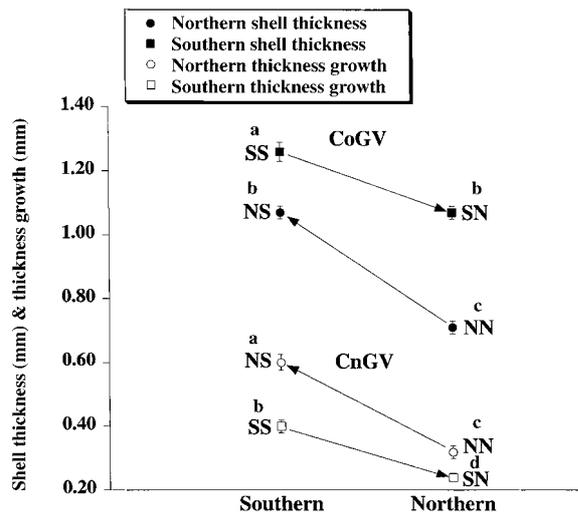


Figure 5. (Top) Adjusted shell thickness ( $\pm$ SE) and (bottom) adjusted shell thickness growth ( $\pm$ SE) for *Littorina obtusata* reciprocally transplanted between a northern and southern site in the Gulf of Maine for 90 days. NN = north to north; NS = north to south; SS = south to south; SN = south to north. Variation in shell thickness shows a cogradients pattern (CoGV) whereas variation in shell thickness growth shows a countergradient pattern (CnGV). All data are least squares adjusted means from ANCOVA. Shell length was the covariate for shell thickness, initial shell thickness was the covariate for shell thickness growth. Groups not sharing a common letter are significantly different (all  $p < 0.01$ ).

those at their native site (Figure 5). In addition, northern snails in the south and southern snails in the north produced shells of identical thickness despite the 75% difference in shell thickness between native northern and southern snails. As for green crab cues, northern snails exhibited nearly twice the shell thickness plasticity of southern snails in response to different water temperatures (see Table 4 in Trussell, 2000a). The greater plasticity of northern snails in response to water temperature may partly reflect countergradient selection for more rapid shell growth under less favorable conditions (see below).

Given the considerable plasticity in shell thickness in response to both water temperature and green crabs, it became important to determine the relative contributions of these to shaping latitudinal clines in shell thickness within the GOM. To address this issue, another reciprocal transplant experiment was conducted in the field between a northern and southern site. In addition, within each transplant location, snails were experimentally exposed to the presence and absence of green crab cues. The results were complex (see Trussell & Smith, 2000), but they strongly suggest that the effects of water temperature and green crab cues

on *Littorina obtusata* shell thickness were similar in magnitude. While other factors, such as differences in growth rate, are likely operating in this system (see Kemp & Bertness, 1984), it seems that both predator cues and water temperature have an important role in producing latitudinal patterns of shell thickness variation.

#### *The importance of cogradients and countergradient variation to understanding patterns of geographic variation*

Because the covariance between genetic and environmental influences on phenotypes can modulate the expression of phenotypic variation, a complete understanding of geographic variation requires determining whether these influences covary in the same (cogradients) or in opposing (countergradient variation) directions (see Conover & Schultz, 1995). The nature of the covariance relationship between genetic and environmental influences can determine the presence or absence of clinal variation. Evaluating this covariance relationship requires reciprocal transplant or common garden experiments with individuals from populations that characterize the environmental gradient of interest (e.g., changing latitude or altitude).

Cogradients variation typically leads to observable phenotypic variation across environmental gradients because environmental effects intensify the effects of genetic influences on phenotypes (i.e., a positive covariance). Figure 6(a) provides a graphical illustration of cogradients phenotypic variation from a reciprocal transplant experiment. The phenotypic differentiation among geographically separated populations is revealed by comparing phenotypes of individuals raised in their native environments ( $N_1$  v.s.  $N_2$ ). In addition, with cogradients variation the phenotypes of transplanted individuals ( $T_1$  vs.  $T_2$ ) converge towards native phenotypes ( $T_1 \rightarrow N_2$ ;  $T_2 \rightarrow N_1$ ).

Countergradient variation (Figure 6(b)) leads to little or no phenotypic differentiation among native phenotypes ( $N_1$  v.s.  $N_2$ ) because genetic and environmental effects on phenotypes oppose one another across the environmental gradient (i.e., a negative covariance). In addition, the phenotypes of transplanted organisms diverge from those of native phenotypes ( $T_1$  v.s.  $T_2$ ). Importantly, if countergradient patterns occur, then considerable genetic differentiation may exist among geographically separated populations despite the absence of phenotypic differentiation across the environmental gradient.

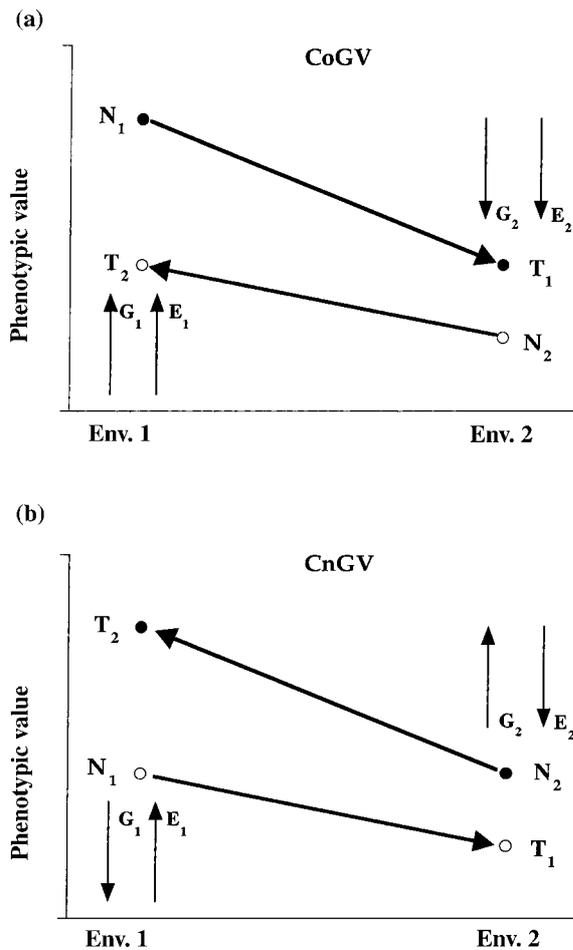


Figure 6. (a) Graphical illustration of cogradients and (b) countergradients variation. Data from the reciprocal transplant with *Littorina obtusata* from the northern and southern GOM (Figure 5) conform well to this conceptual model. Arrows with G and E refer to the direction of genetic and environmental influences on phenotypes within their respective environments. T = transplant, N = native. See text for further explanation (adapted from Conover & Schultz, 1995).

To date, examples of cogradients variation have been confined to morphological traits, whereas countergradients variation is typically found in life history or physiologically-based traits (Conover & Schultz, 1995). It is thus not surprising that most examples of countergradients variation involve temperature effects on traits such as growth in populations across altitude (Levins, 1969; Berven, Gill & Smith-Gill, 1979) or latitude (Dehnel, 1955; Ament, 1979; Parsons, 1997; Trussell, 2000a).

Countergradients variation challenges the assumption that the absence of clinal variation reflects genetic similarities among populations. Considering environ-

ment:genetic covariance relationships has been helpful in understanding phenotypic variation in several taxa, including fruit flies (Levins, 1968), frogs (Berven, Gill & Smith-Gill, 1979), fish (Conover & Present, 1990), and more recently, intertidal snails (Parsons, 1997; Trussell, 2000a) and salmon (Craig & Foote, 2001).

As mentioned above, water temperature appears to exert a strong influence on *Littorina obtusata* shell thickness (also see Trussell, 2000a). Snails reciprocally transplanted between colder northern waters and warmer southern waters exhibited a cogradients pattern in shell thickness (Figure 5). Northern snails transplanted to a southern site produced thicker shells than controls raised at their native site, whereas southern snails transplanted to a northern site produced thinner shells than controls raised at their native site. Snails also exhibited a cogradients pattern in shell length growth (data not shown). Northern snails transplanted to the southern site grew less in terms of shell length than snails raised at their native site whereas southern snails transplanted to the northern site grew more than southern snails raised at their native site. These results are not surprising given that there is a maximum limit to the rate of calcification (Palmer, 1981, 1992). Snails growing faster in shell length are generally thinner than slowly growing snails (Kemp & Bertness, 1984) because calcium carbonate devoted to linear translation of the shell limits the amount of calcium carbonate available for shell thickening.

Interestingly, the cogradients pattern in final shell thickness was accompanied by a countergradients pattern in growth of shell thickness (Figure 5) and shell mass. Northern and southern snails raised at their native locations exhibited remarkably similar rates for both forms of deposition. However, northern snails transplanted to warmer southern waters showed the highest rates of shell deposition, outgrowing even southern snails in their native environments. In fact, despite the inverse relationship between shell thickness growth and shell length growth, northern snails transplanted to the southern site grew more in shell thickness and shell length than southern snails in their native habitat. Despite the rapid growth of northern snails in southern waters, their final shell thickness was still less than that of southern snails raised at the same location. This difference likely reflects the fact that juvenile southern snails were already considerably thicker than juvenile northern snails at the beginning of the experiment.

Neither the thin shells typical of northern snails nor the relative paucity of green crabs in the northern GOM would suggest that northern snails are capable of such rapid deposition. If anything, the thin shells of northern snails suggest that selection has favored thin-shelled genotypes in these environments because the green crab is less common compared to the southern GOM. However, in the northern GOM the evolution of genotypes with increased deposition rates also may be important in offsetting the negative impacts of colder water on shell deposition and maintenance. Without a reciprocal transplant we would not have been able to determine that despite colder waters at their native site, northern snails are able to deposit similar amounts of shell material to southern snails raised in their warmer native waters. Thus, in the colder waters of the northern GOM there must be strong selection for efficient  $\text{CaCO}_3$  deposition. When northern snails are placed in warmer southern waters and released from this environmental constraint, they are able to deposit shell material much more quickly. Northern snails transplanted to the southern site grew 50% more in terms of shell thickness and 76% more in terms of shell mass than southern snails raised at the southern site (Trussell, 2000a). The thinner shells in northern waters suggest that the environmental effects of colder waters on shell form constrain the genetic potential for increased deposition.

It is important to remember, however, that the relative absence of crab predators in the northern GOM coupled with life history trade-offs accompanying thicker shells (Trussell, 2000a,b) are also likely influencing shell production. Thus, the thinner shells of northern snails probably reflect the combined effects of water temperature and life history trade-offs associated with shell thickness.

The patterns observed for shell thickness and thickness growth, and others involving countergradient variation, illustrate how initial perceptions of phenotypic pattern can be misleading and that proper interpretation of geographic variation requires a thorough understanding of the covariance relationship between genetic and environmental influences on phenotypic variation. Knowledge of whether traits exhibit cogeographic or countergradient variation can greatly improve our understanding of how genetic and environmental factors shape patterns of geographic variation.

However, the presence of countergradient variation is puzzling because one would expect superior genotypes (i.e., those for faster growth in northern snails)

to spread through the population and become fixed, eliminating the countergradient pattern (Conover & Schultz, 1995). Although this scenario is intuitively appealing, it is unlikely to apply to organisms having limited dispersal (such as *Littorina obtusata*) or where there are trade-offs between the countergradient trait and other fitness-related traits (Conover & Schultz, 1995). Clearly, much remains to be learned about cogeographic and countergradient variation and its importance to understanding the presence and absence of geographic variation and, perhaps most importantly, its evolutionary significance.

#### *Phenotypic plasticity and trade-offs*

The idea that trade-offs accompany inducible defenses is a central tenet of plasticity theory (Stearns, 1989; DeWitt, Sih & Wilson, 1998; Tollrian & Harvell, 1999). If such trade-offs did not exist, one would expect organisms to produce permanent defenses or constitutive morphologies in general. A consistent result of studies on plasticity in marine gastropods is that inducible changes in traits such as shell thickness are accompanied by reductions in snail body mass (Palmer, 1990; Trussell, 2000a, b; Trussell & Smith, 2000; Trussell & Nicklin, in press). Because snail fecundity is often a positive function of body size (Spight & Emlen, 1976; Palmer, 1983), these trade-offs are likely to have important implications for life history evolution. In studies with *Littorina obtusata*, the magnitude of trade-offs is directly proportional to the amount of induced thickening (Trussell & Nicklin, in press). Although these trade-offs may be partly tied to potential energetic costs associated with depositing thicker shells, most research on this subject suggests that trade-offs in body mass are due to the architectural constraints uniquely associated with living inside a shell. This hypothesis is certainly consistent with the close correspondence between the amount of shell thickening and the reductions in body mass accompanying it. These constraints arise because there is a maximum rate at which calcification can occur (Palmer, 1981, 1992). Hence, snails depositing thicker shells must do so at the expense of linear translation of the shell. In addition, because body growth cannot proceed ahead of the advancing shell margin, the deposition of thicker shells will limit age-specific body growth rate and size. Thus, thick-shelled snails have less internal volume available for body growth than thin-shelled snails of similar size and shape. The presence of these architectural constraints may be a

primary reason underlying the evolution of plasticity in gastropod shell form.

DeWitt, Sih and Wilson (1998) suggested that natural selection should act to minimize the impact of trade-offs. For example, for a given amount of predator-induced shell thickening, those genotypes paying the least amount of trade-off (body mass) should be favored versus those paying more. DeWitt, Sih and Wilson (1998) also proposed that responses to selection may explain why trade-offs are often difficult to detect experimentally. However, trade-offs appear to be commonplace in molluscan taxa. Considering the green crab-*Littorina obtusata* system, one would expect the magnitude of trade-offs to be less for southern versus northern snails because selection by green crabs has been acting on shell thickness and body mass reaction norms for a longer time in the southern GOM. However, recent data (Trussell & Nicklin, in press) reveal that the scaling between shell thickness and body mass is similar among northern and southern snails. In addition to explaining why plasticity in marine gastropod shell form has evolved, architectural constraints may potentially explain the inability of natural selection to produce geographic differentiation in the amount of trade-off.

## Summary

Intertidal snails exhibit pronounced geographic variation across a variety of spatial and temporal scales. Studies of morphological variation have revealed that it represents contemporary as well as historical responses to several interdependent environmental gradients including wave action, predation, temperature, and various forms of physiological stress. Conventional interpretations of this variation have typically invoked selection operating on genetically controlled traits. We have tried to show that similar morphological changes of similar magnitude can be produced plastically. Consequently, we should not uncritically accept the role of selection in producing either geographic or temporal patterns of phenotypic variation. Of course, we recognize that much of the variation will undoubtedly be under genetic control. This is even apparent in those studies documenting plasticity – populations from different wave exposure regimes, predation intensities, or different latitudes are clearly genetically different. Nevertheless, the possibility that plasticity can produce similar changes forces us to be prudent and conservative in

interpreting these patterns. Without experimental verification, we should not conclude that morphological differences reflect genetic differences or the operation of selection. The fact that many of these crucial traits are plastic is also important for understanding the evolution of intertidal organisms because as alluded to earlier, plasticity can have such profound effects on the direction, dynamics and outcome of evolution.

The most well-studied feature of morphological variation in intertidal snails is shell thickness. We can use this as a model for understanding the forces that shape morphological variation in intertidal organisms. Work over the past 20 years suggest that variation in shell thickness represents a complex interplay between genetic and environmental influences integrated across several space and time scales. Populations from different wave-exposure regimes and thus predation intensity, exhibit clear genetic differences in shell thickness. However, experiments have shown that changes in shell thickness can also be induced plastically by exposing snails to crab exudates, crushed conspecifics, different flow regimes or different water temperatures. Plastic increases in shell thickness, in turn, produce decreases in growth rate and body mass. Because both of these traits influence fecundity, the plasticity can modulate various life history traits and potentially maximize fitness in this highly heterogeneous environment. Knowledge of these tradeoffs will be crucial for understanding their evolution.

Reciprocal transplant experiments revealed that genotypes are often nonrandomly distributed among shores differentially exposed to wave action (temperature or predation) creating cogradient or countergradient patterns of variation. Because this can increase or decrease phenotypic variation along an environmental gradient (Conover & Schultz, 1995), morphological differences are not indicative of genetic differences or the intensity of selection. In some cases, there are genotype x environment interactions, such that the magnitude of the plastic response to specific environmental changes varies among populations (genotypes). The G x E interactions suggest that genetic variation in plasticity exists and can be acted upon by natural selection. Plasticity itself may be evolving in response to changes in the spatial and temporal variation in the environment. For example, in the Southern GOM crab predation appears to be less spatially variable and plasticity is less (Trussell, 2000a, b). This suggests that both the traits and their

plasticity are being shaped by the nature, scale and intensity of selection.

An intriguing question is why these snails maintain plasticity to wave action, predation and especially water temperature. Most of the species we have considered have limited lifetime dispersal ability. Their young emerge as benthic juveniles from attached egg capsules and their lifetime ambit is typically within a few tens of meters from where they emerge (Etter, 1989). One might expect that with such limited dispersal potential snails would quickly adapt to local selective pressures. The retention of a plastic response may imply that these snails are perhaps more dispersive than might be predicted from their life histories. This seems reasonable for wave action and predation because they can be highly variable even over small spatial scales (tens of meters). Snails could easily crawl from one exposure regime to another and thus be subjected to different hydrodynamic forces and predation intensities. However, this seems less tenable for water temperature, which changes over much larger spatial scales (hundreds of kilometers). Instead, plasticity in shell deposition rates as well as means attained over certain periods of experimental time may simply reflect temperature effects on the biochemistry of CaCO<sub>3</sub> precipitation and dissolution.

Plasticity is an important mechanism for mitigating the effects of a heterogeneous environment. For intertidal snails, as well as many other intertidal organisms (Barnacles: Lively, 1986; Sponges: Palumbi, 1984; Mussels: Leonard, Bertness & Yund, 1999; Crabs: Smith & Palmer, 1994; Macroalgae: Toth & Pavia, 2000), plasticity in critical traits is well documented, appears to be adaptive and may be essential for persistence in this highly variable environment. Although the plasticity has been quantified, we still need to improve our understanding of how it affects the direction, dynamics or outcome of evolution.

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