doi:10.1111/j.1420-9101.2006.01171.x

Phenotypic plasticity in two marine snails: constraints superseding life history

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Keywords:

dispersal strategy;
grain size;
Littorina littorea;
Littorina saxatilis;
local adaptation;
phenotypic plasticity;
rocky shore.

Abstract

In organisms encountering predictable environments, fixed development is expected, whereas in organisms that cannot predict their future environment, phenotypic plasticity would be optimal to increase local adaptation. To test this prediction we experimentally compared phenotypic plasticity in two rocky-shore snail species; *Littorina saxatilis* releasing miniature snails on the shore, and *Littorina littorea* releasing drifting larvae settling on various shores, expecting *L. littorea* to show more phenotypic plasticity than *L. saxatilis*. We compared magnitude and direction of vectors of phenotypic difference in juvenile shell traits after 3 months exposure to different stimuli simulating sheltered and crab-rich shores, or wave-exposed and crab-free shores. Both species showed similar direction and magnitude of vectors of phenotypic difference with minor differences only between ecotypes of the nondispersing species, indicating that plasticity is an evolving trait in *L. saxatilis*. The lack of a strong plastic response in *L. littorea* might be explained by limits rather than costs to plasticity.

Introduction

Phenotypic plasticity, i.e. induced changes resulting in different phenotypes in different environments, is today recognized as an important evolutionary mechanism that modulates inherited differences of individuals in a wide range of organisms (West-Eberhard, 1989; Brönmark & Miner, 1992; DeWitt, 1998; Scheiner, 1998; Pigliucci, 2001). Although even if a species' capacity for phenotypic plasticity is in itself under genetic control, either directly or indirectly (Via *et al.*, 1995), we cannot, *a priori*, assume that phenotypic plasticity is always an adaptive response under natural selection (Langerhans & DeWitt, 2002).

If there were no costs or limitations to phenotypic plasticity, completely plastic phenotypes would always be the optimal solution for local adaptation. However, constraints to complete plasticity result in essentially three main strategies that organisms can adopt for living in heterogeneous environments: (i) plasticity, (ii) heritable variation in traits (genetic polymorphism), and (iii) a fixed generalist phenotype more or less suitable over a broad range of habitats (Scheiner, 1998). Which strategy is adopted by an individual species is likely related to costs and limitations of the different strategies, as well as to life-history characteristics and temporal and spatial scales of habitat variation.

Although natural selection acts within the limits of available genetic variation and over a time frame of several generations, a plastic phenotype provides a within-generation response to a variable environment, which should be a benefit for individuals living in spatially or temporally heterogeneous environments. However, the expression of optimal phenotypes depends on the inherited capacity for nongenetic regulation of traits, which in turn is constrained by a variety of costs and limits (Pigliucci, 1996, 2001; DeWitt *et al.*, 1998).

An organism's ability to respond to a heterogeneous environment by a genetic polymorphism, a fixed phenotype or phenotypic plasticity, depends also on the scale of the environmental variation in relation to the scale of

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dispersal of the organism: the grain-size (Levins, 1968). If the grain-size of the environmental variation is larger than the organism's range of dispersal (a coarse-grained environment), selection would favour genetic adaptation to local conditions (Levins, 1968; Pigliucci, 2001). If, on the other hand, the grain-size of the environment is less than the typical dispersal range of an organism (a fine-grained environment), genetic adaptation to one of several habitat types is not optimal. In an organism repeatedly encountering different environments within its lifetime, a fixed general phenotype is often expected to evolve (Levins, 1968). If, instead, an organism changes the dispersal potential over its lifetime, for example, having a wide dispersal during the larval stage experiencing the environment as fine-grained whereas a sedentary life during the adult stage experiencing a coarse-grained environment, phenotypic plasticity to increase local adaptation is, in principal, the best evolutionary strategy (Van Tienderen, 1991; DeWitt, 1998). Indeed, if little further migration takes place after settlement, the optimal reaction norm should be reached through plasticity (de Jong & Behera, 2002). However, costs and limits of plasticity might obstruct such a development (DeWitt *et al.*, 1998).

Many marine benthic animals and plants, as well as many terrestrial plants, have a life history that includes long-range post-zygotic dispersal followed by sessile or sedentary juvenile and adult stages. Still, surprisingly little attention has been paid to unravelling the evolution of phenotypic plasticity in relation to, for example, life history of species (Pigliucci, 2001). The purpose of the present investigation was to test if perceived grain-size of species influences the degree of phenotypic plasticity in species with restricted post-settlement migration. For this purpose we chose a pair of closely related marine rocky shore gastropods. Adult individuals of both species live a sedentary life remaining within the same rocky shore habitat throughout life (Janson, 1983; Erlandsson *et al.*, 1998). The two species have different larval dispersal strategies: one broadcasting egg and larvae that disperse with water currents during 4–6 weeks, the other giving birth to miniature crawl-away juveniles remaining in the close vicinity of the parents (Reid, 1996). The rocky shore is a highly heterogeneous habitat and both species are found in wave-exposed, crab-free rocky shores as well as in sheltered boulder, crab-rich shores, although *Littorina littorea* is much less common in the former type of shore than in the latter. Larvae of the broadcasting species (*L. littorea*) encounter different types of rocky-shore habitats more or less at random, thus experiencing a fine-grained environment, whereas adults, having settled in one type of habitat, experience a coarse-grained environment. Consequently, we predict that this species would benefit from having a more flexible phenotype than the species with no larval dispersal (*Littorina saxatilis*), in which juveniles almost always grow up in the habitat of their parents. In our study we tested this prediction by experimentally raising juveniles of both species under different conditions, simulating the two main habitats they experience in nature: a wave-exposed rocky shore without crabs and a sheltered boulder shore with crabs.

Selection does not usually act on single traits, either when traits are correlated, or when there are trade-offs between competing functions (Reznick & Travis, 1996), thus a multivariate analysis is more informative than multiple analyses of traits that are mostly not independent. In studies of phenotypic plasticity, the norm of reaction (Schmalhausen, 1949) – the set or function that describes the change in phenotype between environments – can be considered with vectors of phenotypic change in the multivariate trait space, having both the properties of magnitude (amount of phenotypic change) and direction (covariation of phenotypic traits). To interpret the biological relevance of a multivariate plastic response (e.g. morphology), one must consider both of these attributes of phenotypic change. For this purpose we examined different vectors of phenotypic difference in both magnitude and direction in multivariate data spaces (e.g. Klingenberg & Leamy, 2001; Adams, 2004). We used this method to evaluate measurements indicating the ontogenetic development of shell morphology in juveniles of the two species raised under different environmental conditions.

Material and methods

The organisms

The marine gastropod genus *Littorina* is found throughout the world in the littoral zone, occupying a wide range of shore environments (Reid, 1996). *Littorina littorea* and *L. saxatilis* largely overlap in both macro- and micro-geographical distribution, for example, being common in boulder and rocky shores of west Sweden. Both species show intra-specific variation in shell traits, but *L. littorea* is much less variable among shore habitats than is *L. saxatilis* (Behrens Yamada, 1987, 1989; Janson, 1987). *Littorina saxatilis* being characterized by a strong genetically determined shell polymorphism resulting in substantial differences in shell traits such as size, shell thickness, aperture area, etc. between different rocky shore environments. Indeed, this polymorphism has led to descriptions of various ecotypes of *L. saxatilis*, each being present in a specific microhabitat, such as, wave-exposed rocky shores, boulder beaches and submersed lagoons (Reid, 1996; Johannesson, 2003). Sometimes the same shore is occupied by different ecotypes at different tidal levels (Johannesson *et al.*, 1993; Hull *et al.*, 1996).

We included two Swedish ecotypes; the E-morph (exposed) and the S-morph (sheltered) in the present study. The E-morph is adapted to rocky shores with strong wave action, having a small size, a thin shell and a large aperture and foot. In contrast, the S-morph is

adapted to boulder shores with crabs, being large, with a thick shell and a relatively small aperture (Janson, 1982a; Johannesson & Johannesson, 1996). Close subpopulations of different ecotypes are genetically very similar in allozyme and micro-satellite loci (Janson & Ward, 1984; Panova *et al.*, in press). Indeed earlier genetic studies show that within a local area, such as an island, subpopulations of contrasting ecotype most probably have evolved from a common ancestral population and that snails of similar ecotype from separate areas evolve by parallel evolution (Johannesson, 2001; Rolán-Alvarez *et al.*, 2004; Panova et al., in press). A dominating part of the shell size and shape differences is inherited (Janson, 1982b; Johannesson & Johannesson, 1996) and a result of strong divergent selection between habitats (Janson, 1983; Rolán-Alvarez *et al.*, 1997). The strong genetic polymorphism in shell size and shape in *L. saxatilis* over rocky shore environments thus suggests that locally adapted shell traits are indeed an advantage in the heterogeneous rocky shore environment. It also indicated that large phenotypic differences could be produced suggesting no major mechanical constraints to extensive shell variation in this genus.

Experimental design

Small juveniles of each group (*L. littorea* and E and S of *L. saxatilis*) were collected in the surrounding of Tjärnö Marine Biological Laboratory, Swedish west coast. All groups were sampled from various locations in the area and juveniles were pooled in the experiment to include any existing variation between local sites. We conducted a common garden experiment where juveniles of each group were either exposed to artificial wave action or to effluent cues associated to predation. Thus the four treatments were: (i) water borne effluent from a predator (*Carcinus maenas*), (ii) water borne effluent from crushed conspecifics simulating any kind of predation, (iii) wave action produced by rocking the table of the aquarium, and (iv) a 'no-treatment' group. The rocking table was connected to an electrical engine and artificial wave action was generated in 2-h intervals 24 h per day. All treatments were raised in out-door aquaria provided with flow-through seawater and a natural flora of micro algae. All snails had the size of <2 mm at the start of the experiment. Each group and treatment was replicated in three aquaria with at least 50 snails in each. The experiments were performed during summer 2004 and juveniles grow for 90 days in each treatment.

Morphometric and statistical analyses

We compared phenotypic plasticity in shell shape, described by Cartesian coordinates of anatomical landmarks. Shell shape was analysed using landmark based geometric morphometrics (Bookstein, 1991; Rohlf & Marcus, 1993). To digitize the landmarks, we used a dissecting microscope with a camera lucida and a digitizing table together with the program DS-DIGIT (Slice, 1994) to collect eight anatomical landmarks (see Fig. 1 in Hollander *et al.*, 2005). Each snail was positioned such that the aperture facing upward (Hollander *et al.*, 2005). The digitized landmark configurations were then subjected to a generalized Procrustes analysis (GPA; Rohlf & Slice, 1990) to remove the effects of specimen size, position and orientation in the digital images. The aligned landmark configurations produced by GPA were used to generate shape variables as partial warp scores from a thin-plate spline analysis (TPS) and two uniform scores. These variables can be used in multivariate analyses of shape variation and covariation with other variables (see, e.g. Adams, 2004; Collyer *et al.*, 2005).

Comparison of overall shapes

We considered if there was distinction among the various species and ecotype shapes (i.e. they occupied different regions of shape space) by performing a principal component analysis (PCA) on the set of shape variables, including partial warp scores and the uniform components. We projected the means of experimental

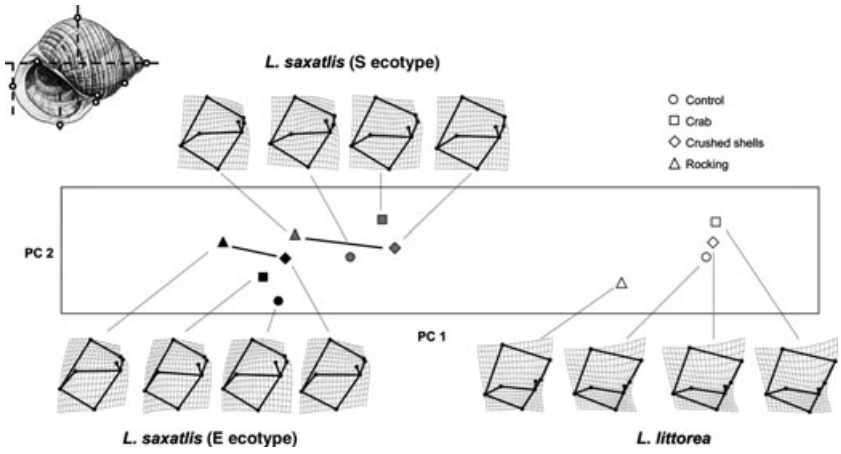

Fig. 1 Principal component plot of shape variation for *Littorina*. The first two principal components (PC) are shown (with axis aspect preserved), accounting for 61.8% of the overall shape variation. Deformation grids, scales 3×, are shown for each value, which are least-squares treatment means projected onto the first two PCs. Solid lines between crushed shells and rocking treatments highlight the parallel vectors of phenotypic difference for E and S ecotypes of *Littorina saxatilis*.

treatments for each group (*L. littorea*, E and S ecotype of *L. saxatilis*) onto the first two principal components, which accounted for 61.8% of the total shape variation. We used this visualization to consider if obvious group-specific differences in shape warranted the preservation of within-group shapes in subsequent statistical analyses (see below). Clear separation of groups (i.e. intra-group environmental distances are shorter in shape space compared with inter-group distances) would suggest that group-specific shape differences be held constant. Further, distinction between the ecotypes of *L. saxatilis* would provide evidence for habitat-linked phenotypic differentiation.

Assessment of phenotypic plasticity and within-environment shape variation

To consider whether significant phenotypic plasticity was observed in the experiment, we performed multivariate analysis of variance (MANOVA) on shape to test for heterogeneity of experimental treatment means for each group (*L. littorea*, E and S ecotype of *L. saxatilis*). Although MANOVA can indicate if significant phenotypic plasticity exists, other analyses are needed to consider differences in relative measures of phenotypic plasticity among groups. We used as an overall measure of the magnitude of phenotypic plasticity the weighted disparity among experimental treatment means (*sensu* Foote, 1993; Zelditch *et al.*, 2003). We likewise calculated the within-treatment (D_W) and pooled within-treatment (D_{PW}) disparities to consider the shape variation within-environmental treatments (See Appendix 1 for statistical details). The same D_A can be achieved by different arrangement of treatment means. Thus, pair-wise vectors of phenotypic change were statistically compared between and within ecotypes for the six possible vectors that describe differences in four treatment means. These vectors were compared in terms of two important attributes, magnitude and direction, which describe the amount of phenotypic plasticity and direction of phenotypic change based on trait (shape variable) covariation, respectively (see, e.g. Klingenberg & Leamy, 2001; Adams, 2004). A description of the test statistics calculated for these measures, plus the permutation procedure used to evaluate them is provided in Appendix 1.

Results

We found consistency of shape variation among experimental treatments and among group positions in shape space, i.e. the same treatment tended to change shape in a similar way in both ecotypes of *L. saxatilis* and in *L. littorea*. Nevertheless, the principal axis of shape variation (PC 1) revealed distinction of the E and S ecotypes of *L. saxatilis*, and a more pronounced distinction of *L. saxatilis* from *L. littorea* (Fig. 1).

Both the two ecotypes of *L. saxatilis* and *L. littorea* revealed phenotypic plasticity, i.e. significant differences among treatment means (MANOVA: *L. saxatilis* E ecotype: Wilks' $\Lambda = 0.625$, Approximate $F_{36,910.75} = 4.355$; $P < 0.0001$; *L. saxatilis* S ecotype: Wilks' $\Lambda = 0.541$, Approximate $F_{36,1014.2} = 6.504$; $P < 0.0001$; *L. littorea*: Wilks' $\Lambda = 0.569$, Approximate $F_{36,1020.1} = 5.956$; $P < 0.0001$). However, the magnitude of the overall phenotypic plasticity (Table 1, among-treatment values) was significantly lower for the E ecotype than for the S ecotype (Table 2). *Littorina littorea* was intermediate in its measure of overall phenotypic plasticity and did not significantly differ from either the E or S ecotype of *L. saxatilis*. Within treatments (Table 1, pooled within-treatment values), the E ecotype demonstrated significantly greater phenotypic variation than either the S ecotype or *L. littorea* (Table 2).

The morphological distinction between the groups of E and S along the PC 1 axis reflected the inherited differences present between the two ecotypes (Fig. 1). Additionally, there were directional shape changes along PC 1 between crab/crushed shell treatments and rocking treatments, within ecotypes, that were concordant with the distinction between S and E morph, respectively (Fig. 1). This indicated that the plastic responses tended to mimic the inherited responses of S and E ecotypes presumably created by divergent natural selection.

Table 1 Measures of shape disparity from Procrustes residuals for the two ecotypes of *Littorina saxatilis* and *Littorina littorea*.

Disparity	*Littorina saxatilis* E ecotype	*Littorina saxatilis* S ecotype	*Littorina littorea*
Control	**0.00601***	0.00358	0.00369
Crab	**0.00314†**	0.00372	0.00332
Crushed shells	0.00408	0.00355	0.00350
Rocking	0.00415	**0.00322†**	0.00380
Pooled within-treatment	0.00425	0.00349	0.00355
Among-treatment	0.00018	0.00029	0.00023

Values in bold represent significant departures from random shape disparity. See Appendix 2 for test details.
*Indicates significantly greater disparity.
†Indicates significantly less disparity.

Table 2 *F*-ratios* for comparisons of among-treatment disparity (above diagonal) and pooled-within treatment disparity (below diagonal).

	E ecotype	S ecotype	*Littorina littorea*
E	–	**1.797**	1.433
S	**1.219**	–	1.254
L l	**1.198**	1.018	–

Values in bold are significant ($P < 0.05$).
*36 d.f. among treatments for each group; E: 3864 d.f. within; S: 4308 d.f. within; L l: 4284 d.f. within.

Within treatments, both ecotypes of *L. saxatilis* showed departures from random shape disparity; the E ecotype being more variable in shape in the control environment and less variable in the crab environment than expected by chance, whereas the S ecotype being less variable in the rocking environment (Table 1). Thus the reduced variation in shape for each ecotype was in an experimental environment that mimicked the natural environment of the other ecotype. In *L. littorea* shape disparity within treatments did not deviate from random (Table 1).

Differences in the amount of phenotypic plasticity between treatment means were variable for each group and not consistent over ecotypes of *L. saxatilis* or over species (Table 3). The vector of phenotypic difference between crushed shell and crab treatments (V 4) was small in magnitude in both ecotypes of *L. saxatilis* and in *L. littorea*, suggesting that both of these stimuli produced similar shell shapes. However, the largest vectors were represented by contrasts that included the experimental treatment that mimicked the natural environment for the E and S ecotypes. For the E ecotype, the largest vector of phenotypic difference was between no-treatment and rocking (V 3) and vectors between rocking and crab (V 5) and rocking and crushed shells (V 6) were similar and intermediate in magnitude. For the S ecotype, vectors between the crab and rocking (V 5) and the crab and no-treatment environments (V 1) were largest in magnitude. In particular, for the E ecotypes of *L. saxatilis*, shape means were most divergent for rocking, as they were for *L. littorea*, suggesting that wave action imposes stronger environmental influence than the presence of crabs (see also Fig. 1).

No vectors of phenotypic difference were significantly correlated within either the E or the S ecotype of *L. saxatilis*. However, three vectors were correlated with each other within *L. littorea*, all involving the rocking treatment [V 3/V 5: vector correlation (VC) = 0.927, $\theta = 22.0\ ^\circ$, $P_{\mathrm{Rand}} = 0.0039$; V 3/V 6: VC = 0.947, $\theta = 18.8\ ^\circ$, $P_{\mathrm{Rand}} = 0.0016$; V 5/V 6: VC = 0.952, $\theta = 17.8\ ^\circ$, $P_{\mathrm{Rand}} = 0.0009$; Appendix 2]. The only vectors that were significantly correlated between groups involved the crushed shells and rocking treatments (V 6: VC = 0.868, $\theta = 29.8\ ^\circ$, $P_{\mathrm{Rand}} = 0.0011$). This result is consistent with the relatively large magnitude of this vector for both the *L. saxatilis* ecotypes and for *L. littorea* (Table 3) and the alignment of the principal axis of shape variation (Fig. 1).

Table 3 Vector magnitudes (d) for pair-wise treatment comparisons within the two ecotypes of *Littorina saxatilis* and *Littorina littorea*.

Littorina saxatilis (E ecotype)		*Littorina saxatilis* (S ecotype)		*Littorina littorea*	
V	d	V	d	V	d
3	0.030 a	5	0.035 a*	5	0.036 a
2	0.024 ab	1	0.034 a*	6	0.032 a
6	0.022 ab	6	0.030 ab	3	0.030 a
5	0.021 ab*	2	0.024 b	1	0.014 b
1	0.019 b*	3	0.021 bc	4	0.011 b
4	0.014 b	4	0.020 bc	2	0.010 b

Vector numbers (V) correspond to the following comparisons: (1) Control/Crab, (2) Control/Crushed shells, (3) Control/Rocking, (4) Crab/Crushed shells, (5) Crab/Rocking and (6) Crushed shells/Rocking. Values are ranked in descending order. Vectors sharing a letter do not significantly differ (see Appendix 2).
*Indicates significant difference of vector magnitude between E and S ecotypes of *Littorina saxatilis*.

Discussion

It is quite obvious from theory and models discussed earlier that in species in which the adults experience a coarse-grained habitat, the dispersal capacity of the larva is crucial; species with no larva are likely to evolve genetic polymorphisms to cope with heterogeneous environments, whereas species with broadcasting larva would benefit from evolution of phenotypic plasticity to adapt to a heterogeneous environment (Levins, 1968; Pigliucci, 2001). Thus, in our study, we predicted that phenotypic plasticity would be an efficient mechanism of local adaptation in the species with a long larval dispersal stage (*L. littorea*) but less important in the species with direct development (*L. saxatilis*). Nevertheless, we found no difference in magnitude or direction of plasticity between the two species.

Earlier studies show that gene flow is restricted over distances of tens of metres and more in *L. saxatilis*, whereas *L. littorea* is hardly differentiated at all over distances of hundreds of km (Janson & Ward, 1984; Janson, 1987; Johannesson, 1992), thus supporting our conclusion of an extremely restricted dispersal in *L. saxatilis* but an effective larval dispersal in *L. littorea* over wide geographic areas. Dispersal potentials other than those expected from life-history traits could thus not be the reason for a bad fit to the predicted pattern of plasticity. Furthermore, the poor dispersal of *L. saxatilis* supports inherited and strongly polymorphic traits, leading to the evolution of remarkably local adapted ecotypes sometimes differing substantially in quantitative traits over less than 20 m (Janson & Ward, 1984; Panova et al., in press). In contrast, *L. littorea* is phenotypically much less variable than *L. saxatilis* over much of its geographic distribution (Behrens Yamada, 1987, 1989; Janson, 1987).

Substantial phenotype differences between the E and S ecotype in the no-treatment aquaria (Fig. 1) support earlier conclusions of large parts of the variation being inherited. Indeed, reciprocal transplantations between habitats and common garden rearing of snails showing pronounced fixed developmental differences between snails from different environments (Janson, 1982b; Behrens Yamada, 1989; Johannesson & Johannesson,

1996). Thus although *L. saxatilis* has a strong genetic polymorphism for a range of shell traits (size, shell thickness, aperture area, spire height, etc.) that increase local fitness (Janson, 1983) the plasticity we observed is likely to further improve local adaptation. Thus we believe that *L. saxatilis* benefits from the combined effect of natural selection and plasticity to evolve locally adapted phenotypes. Studies on other gastropod species lacking a dispersal stage show similarly that adaptive plasticity is often combined with inherited trait differences improving adaptation to contrasting habitats (Appleton & Palmer, 1988; Etter, 1988; Palmer, 1990; Trussell, 1996, 1997a,b).

Littorina littorea, on the other hand, would likely benefit from more plasticity than observed. The rationale is that with a more pronounced plasticity *L. littorea* would be able to produce more extreme phenotypes and in this way increase its range of habitats to include also the most exposed rocky habitats (where the E ecotype of *L. saxatilis* is doing very well, but adult *L. littorea* is rarely found). Today, high numbers of newly settled *L. littorea* is found at wave-exposed rocky shores, but few survive to adult sizes, except for those finding shelter in high-shore rock-pools (personal observation). It seems thus obvious that one or more constraints impede the evolution of a stronger plastic response in this species. DeWitt *et al.* (1998) distinguish between costs and limits to plasticity; a cost being present when a plastic response is produces but to the prize of a lower fitness. As in *L. littorea*, we did not find any plastic response beyond those of *L. saxatilis*, this suggested a limit (rather than a cost) to evolve what would have been an optimal degree of plasticity in *L. littorea*.

Among the four categories of limits to plasticity suggested by DeWitt *et al.* (1998) lag-time of the plastic response seems least likely in this case, as rapid changes in the pattern of shell development is possible even in adult snails of a related taxa upon transplantation to a contrasting environment (Yeap *et al.*, 2001). Information reliability (DeWitt *et al.*, 1998) might, however, be a limit to plasticity in *L. littorea*, in particularly in exposed rocky shores, possibly explaining this species' near absence in these shores. In crab-rich shores, crab effluents are presumably reliable triggers to a plastic response in both *L. littorea* and *L. saxatilis*, but in wave-exposed shores the weather might be calm upon settlement producing no necessary trigger to a plastic response protecting against wave action. Interestingly, Trussell (1997a) found an asymmetric plastic response in foot-size in a related species (*Littorina obtusata*) with more plasticity in sheltered as compared with wave-exposed shores. Experiences from other species suggest that extreme phenotypes are more likely to develop through genetic polymorphisms than through phenotypic plasticity (DeWitt *et al.*, 1998). Indeed, ecotype formation in *L. saxatilis* results in more extreme shell forms than what is found in *L. littorea*. An additional constraint might simply be mechanical constraints as a result of a change in shell form causing a breakpoint in the accretionary growth of the shell (DeWitt *et al.*, 1998).

Notably, growth rate seems tightly linked to shell form in *L. littorea*. Interestingly, earlier studies have shown that slower growth produce more elongated and robust shell forms whereas faster growth produce more globular and thin shells in this species and in a related species (Kemp & Bertness, 1984; Boulding & Hay, 1993). In *L. saxatilis*, however, the genetically controlled development instead links a slow growth with a globular and thin shell in wave-exposed habitats, whereas in crab-rich sheltered habitats rapid growth is combined with elongated and robust shells (Johannesson & Johannesson, 1996). This suggests that the combination of traits promoted by natural selection in either wavy or crab-rich habitats might not be supported by the inherited trait correlation present in *L. littorea*.

Although both ecotypes of *L. saxatilis* revealed plasticity for the experimental treatments, the E ecotype showed significantly less overall plasticity than the S ecotype. Moreover magnitudes of specific vectors of phenotypic difference differed between ecotypes. These observations are interesting in that they suggest that plasticity is evolving differently within local populations of the same species. As earlier studies suggest that the E and S ecotypes of *L. saxatilis* evolve repeatedly by parallel evolution in local sites (e.g. islands), and do not make up evolutionary old and separate lineages (Johannesson, 2001; Rolán-Alvarez et al., 2004; Panova et al., in press), the plasticity differences between the E and S ecotypes suggest high evolvability of the plasticity itself in this species, a somewhat unexpected finding in a species, expected to adapt as far as possible to a specific environment through directional selection.

The largest vectors of phenotypic difference for both E and S were for the treatments that mimicked their home-environments, lending further support to the conclusion that plasticity is tightly coupled to fitness of individuals. Foot size, for example, increased substantially when E ecotypes were exposed to the wave treatment. Phenotypic plasticity increasing foot size when exposed to increased wave-action has earlier been described in *L. obtusata* (Trussell, 1997a) and in *Nucella* spp. (Appleton & Palmer, 1988; Etter, 1988). In contrast, the S ecotype revealed most plastic response in the crab treatment, decreasing aperture area compared with the control treatment. Snails living in crab-rich habitats such as boulder beaches, increase their fitness evolving thicker shells and more pronounced spires but less wide apertures (Johannesson, 1986; Trussell, 1996, 2000).

Both ecotypes exhibited less within-treatment disparity in the treatment mimicking the environment of the other ecotype, suggesting that, for some reason, the plasticity response were in fact more accurate in a habitat the snails seldom experience. An observation to some extent parallel to what is observed in another gastropod

species. *Physella virgata*, a fresh water snail raised together with molluscivorous or nonmolluscivorous fishes, produced similar phenotypic responses for both types of fishes, indicating that plasticity might sometimes be less well focused, probably as a consequence of constraints to the evolution of a perfectly adaptive phenotypic plasticity (Langerhans & DeWitt, 2002).

Thus our conclusion is that life history is not tightly related to magnitude of phenotypic plasticity in the pair of species investigated (*L. littorea* and *L. saxatilis*).

This result is different from that of an earlier investigations of plasticity in littoral gastropods in which two species with contrasting life histories were compared; *Austrocochlea constricta* with a short larval stage and *Bembicium vittatum* with no larval stage. In these species, the broadcasting species shows a more pronounced level of plastic response than the direct developing one upon reciprocal transplantation (Parsons, 1997, 1998). However, although the more plastic species had a short-lived pelagic larva, also this species was genetically subdivided over the scale of the investigation and thus genetic differences contributing to the phenotypic differentiation between environments could be as important as plasticity (Parsons, 1997). In light of these findings we conclude that it seems difficult to generalize about the importance of life history to the evolution of plastic responses from observations of a few species. It is noteworthy that constraints to plasticity can effectively impede the production of an optimal phenotype through plastic development, and limits to evolve plasticity might even preclude adaptation to a potential environment.

Acknowledgments

This project was sponsored in part by National Science Foundation grants DEB-0446758 to DCA and DMS-0091953 (which provided post-doctoral support for MLC), the Swedish Research Council (to KJ), the European Commission (contract EVK3-CT-2001-00048 to KJ) and Göteborgs University Jubileumsfond (to JH). This paper benefited from the comments of two anonymous reviewers.

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Received 30 January 2005; revised 3 April 2006; accepted 9 May 2006

Appendix 1: Statistical details

Assessment of phenotypic plasticity and within-environment shape variation

We used as an overall measure of the magnitude of phenotypic plasticity the weighted disparity among experimental treatment means (*sensu* Foote, 1993; Zelditch *et al.*, 2003). This disparity is calculated by the following formula:

$$D_{\mathrm{A}} = \frac{\sum_{i=1}^{a} n_i (\bar{X}_i - \bar{\bar{X}})^T (\bar{X}_i - \bar{\bar{X}})}{\sum_{i=1}^{a} n_i - 1},$$

where $\bar{X}_i$ is the ith treatment mean for a total of a treatments, $\bar{\bar{X}}$ is the overall ecotype mean, and n_i is the ecotype × treatment sample size. The $(\bar{X}_i - \bar{\bar{X}})^T(\bar{X}_i - \bar{\bar{X}})$ portion of the formula is the squared Euclidian distance (d) of the ith treatment mean from the overall ecotype mean. Thus, large values of D_{A} indicate greater dispersion of treatment means whereas smaller values indicate greater clustering (i.e. lack of phenotypic plasticity). We likewise calculated the within-treatment (D_{W}) and pooled within-treatment (D_{PW}) disparities to consider the shape variation within environmental treatments, using the following formulas:

$$D_{\mathrm{Wi}} = \frac{\sum_{j=1}^{n} (X_{ij} - \bar{X}_i)^T (X_{ij} - \bar{X}_i)}{n_i - 1} \quad \text{and}$$

$$D_{\mathrm{PW}} = \frac{\sum_{i=1}^{a} \sum_{j=1}^{n} (X_{ij} - \bar{X}_i)^T (X_{ij} - \bar{X}_i)}{\sum_{i=1}^{a} n_i - 1}.$$

for j individuals in i treatments. The former describes the amount of shape variation within a particular experimental environment whereas the latter describes the amount of shape variation within all experimental environments. Disparity measures within groups were tested using a permutation procedure (see below). Comparison of D_{A} and D_{PW} between ecotypes of *Littorina saxatilis* is analogous to a Procrustes ANOVA, and were therefore performed with a generalization of the Goodall's (1991) F-test.

Comparison of pair-wise vectors of phenotypic change

For the four experimental treatments in our study, six vectors of difference between treatment means can be described for each group. Each vector has two attributes: a magnitude and a direction in the multivariate data space (M.L. Collyer & D.C. Adams, unpublished data). The magnitude is the Euclidian distance of the vector, $d = [(\bar{X}_i - \bar{X}_j)^T (\bar{X}_i - \bar{X}_j)]^{1/2}$, between treatment means i and j (see, e.g. Adams, 2004). The difference in magnitude of phenotypic plasticity is thus described as the absolute value of difference in Euclidian distances, $|d_{ij} - d_{kl}|$, where k and l correspond to two treatment means, and at least one mean is for a different treatment than those considered for i and j. The difference in vector directions can be inferred from the vector correlation, VC (see Klingenberg & Leamy, 2001), calculated as the inner product of two vectors standardized to unit length:

$$\mathrm{VC} = \left[\frac{(\bar{X}_i - \bar{X}_j)}{d_{ij}}\right]^T \left[\frac{(\bar{X}_k - \bar{X}_l)}{d_{kl}}\right].$$

The angle between vectors, θ, is simply $\cos^{-1}(\mathrm{VC})$. This angle will be small if vectors point in similar directions, 90° if they are orthogonal, and approach 180° if the tend to be in opposite directions. We tested pair-wise differences in vector magnitudes and directions, both within groups and across groups, using a permutation procedure (see below). In our tests, we used the absolute value of VC to create random distributions that were bounded between parallel and orthogonal relationships.

Permutation procedure

For the entire test statistics described, we considered a null model of shape variation where snail ecotype and species differences were preserved. This could be achieved by creating random treatment means within groups using a permutation procedure. Within groups, the significance of D_{A}, D_{PW}, $|d_{\mathrm{ij}} - d_{\mathrm{kl}}|$, and |VC| test statistics was considered by calculating the empirical probabilities of random values equalling or exceeding observed values (i.e. one-tail tests). Observed values were considered significant if the empirical probabilities were less than expected for an experiment-wise error rate of $\alpha = 0.05$ for similar comparisons. To maintain the experiment-wise error rate, we used sequential Bonferroni corrections for pair-wise comparisons (Rice, 1989). However, the significance of each D_{W} was considered with a two-tail test, as small within-treatment variation could indicate an extreme outcome under the null hypothesis of equal within-treatment shape variation, within ecotypes. Among groups, the significance of $|d_{\mathrm{ij}} - d_{\mathrm{kl}}|$ and |VC| test statistics was considered similarly for comparison of analogous vectors.

We tested a total of 144 test statistics with the same permutation method. These tests included 18 tests for disparity, 45 tests to compare vector magnitudes within ecotypes, 18 tests to compare vector magnitudes between ecotypes, 45 tests to compare vector correlations within ecotypes and 18 tests to compare vectors correlations between ecotypes (Appendix 2). This method involved randomly assigning individual snails to experimental treatments, within group blocks, and recalculating all values for 9999 iterations. Along with observed values, this procedure created distributions of 10 000 random values from which the significance of observed values was inferred.

Appendix 2: Results of statistical tests from a permutation procedure with 10 000 random permutations, restricted to individuals within ecotype/species groups. For each test, the type of statistic tested, the ecotypes (E) and comparates (C) involved, the observed values, the probabilities of more extreme random outcomes, and adjusted pair-wise alpha values (using a Bonferroni correction for disparity values and a sequential Bonferroni correction for vector comparisons) are shown. Ecotypes are designated as *Littorina saxatilis* E ecotype (E), *L. saxatilis* S ecotype (S) and *Littorina littorea* (L). Vectors are numbered as in Table 3. Significant values are indicated in bold (as compared with adjusted α).

Test type	E 1	E 2	C 1	C 2	Observed value	$P(X_{\mathrm{Rand}} \geq x)$	$P(X_{\mathrm{Rand}} \leq x)$	α
Among-treatment weighted disparity	E				0.0002	**0.0001**	–	0.0167
Among-treatment weighted disparity	S				0.0003	**0.0001**	–	0.0167

Appendix 2 Continued.

Test type	E 1	E 2	C 1	C 2	Observed value	$P(X_{Rand} \geq x)$	$P(X_{Rand} \leq x)$	α
Among-treatment weighted disparity	L				0.0002	**0.0001**	–	0.0167
Pooled within-ecotype disparity	E				0.0043	1.0000	–	0.0167
Pooled within-ecotype disparity	S				0.0035	1.0000	–	0.0167
Pooled within-ecotype disparity	L				0.0036	1.0000	–	0.0167
Treatment disparity	E		Control		0.0060	**0.0001**	1.0000	0.0042
Treatment disparity	E		Crab		0.0031	1.0000	**0.0001**	0.0042
Treatment disparity	E		Crushed		0.0041	0.8441	0.1560	0.0042
Treatment disparity	E		Rock		0.0041	0.7717	0.2284	0.0042
Treatment disparity	S		Control		0.0036	0.8242	0.1759	0.0042
Treatment disparity	S		Crab		0.0037	0.5954	0.4047	0.0042
Treatment disparity	S		Crushed		0.0036	0.8659	0.1342	0.0042
Treatment disparity	S		Rock		0.0032	0.9978	**0.0023**	0.0042
Treatment disparity	L		Control		0.0037	0.6416	0.3585	0.0042
Treatment disparity	L		Crab		0.0033	0.9810	0.0191	0.0042
Treatment disparity	L		Crushed		0.0035	0.8874	0.1127	0.0042
Treatment disparity	L		Rock		0.0038	0.4397	0.5604	0.0042
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V3	V4	0.0159	**0.0001**	–	0.0034
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V1	V3	0.0104	**0.0036**	–	0.0037
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V2	V4	0.0103	0.0040	–	0.0039
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V3	V5	0.0090	0.0105	–	0.0043
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V4	V6	0.0081	0.0170	–	0.0047
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V3	V6	0.0078	0.0237	–	0.0051
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V4	V5	0.0069	0.0391	–	0.0057
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V2	V3	0.0056	0.0967	–	0.0064
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V1	V4	0.0055	0.1007	–	0.0073
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V1	V2	0.0048	0.1367	–	0.0085
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V2	V5	0.0034	0.3805	–	0.0102
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V1	V6	0.0026	0.4760	–	0.0127
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V2	V6	0.0022	0.5113	–	0.0170
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V1	V5	0.0014	0.6656	–	0.0253
$\lvert d_1 - d_2 \rvert$ within ecotype	E		V5	V6	0.0012	0.7150	–	0.0500
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V4	V6	0.0204	**0.0001**	–	0.0034
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V4	V5	0.0248	**0.0001**	–	0.0037
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V3	V4	0.0187	**0.0001**	–	0.0039
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V2	V6	0.0216	**0.0001**	–	0.0043
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V2	V5	0.0260	**0.0001**	–	0.0047
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V2	V3	0.0199	**0.0001**	–	0.0051
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V1	V6	0.0178	**0.0001**	–	0.0057
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V1	V5	0.0222	**0.0001**	–	0.0064
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V1	V3	0.0161	**0.0001**	–	0.0073
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V3	V5	0.0061	0.0383	–	0.0085
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V5	V6	0.0045	0.1246	–	0.0102
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V1	V2	0.0038	0.1858	–	0.0127
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V1	V4	0.0026	0.3631	–	0.0170
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V3	V6	0.0017	0.5641	–	0.0253
$\lvert d_1 - d_2 \rvert$ within ecotype	L		V2	V4	0.0012	0.6671	–	0.0500
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V1	V3	0.0130	**0.0001**	–	0.0034
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V1	V4	0.0134	**0.0001**	–	0.0037
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V3	V5	0.0138	**0.0001**	–	0.0039
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V4	V5	0.0142	**0.0001**	–	0.0043
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V1	V2	0.0100	**0.0004**	–	0.0047
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V2	V5	0.0108	**0.0011**	–	0.0051
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V4	V6	0.0096	**0.0016**	–	0.0057
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V3	V6	0.0092	**0.0019**	–	0.0064
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V2	V6	0.0062	0.0352	–	0.0073
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V5	V6	0.0046	0.1124	–	0.0085
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V2	V4	0.0033	0.2387	–	0.0102
$\lvert d_1 - d_2 \rvert$ within ecotype	S		V1	V6	0.0038	0.2527	–	0.0127

Appendix 2 Continued.

Test type	E 1	E 2	C 1	C 2	Observed value	$P(X_{Rand} \geq x)$	$P(X_{Rand} \leq x)$	α
$\|d_1 - d_2\|$ within ecotype	S		V2	V3	0.0030	0.2881	–	0.0170
$\|d_1 - d_2\|$ within ecotype	S		V1	V5	0.0008	0.7783	–	0.0253
$\|d_1 - d_2\|$ within ecotype	S		V3	V4	0.0003	0.9119	–	0.0500
$\|d_1 - d_2\|$ between ecotype	E	L	V5		0.0154	**0.0001**	–	0.0028
$\|d_1 - d_2\|$ between ecotype	S	L	V1		0.0197	**0.0001**	–	0.0030
$\|d_1 - d_2\|$ between ecotype	S	L	V2		0.0135	**0.0003**	–	0.0032
$\|d_1 - d_2\|$ between ecotype	E	S	V5		0.0137	**0.0005**	–	0.0034
$\|d_1 - d_2\|$ between ecotype	E	S	V1		0.0143	**0.0007**	–	0.0037
$\|d_1 - d_2\|$ between ecotype	E	L	V2		0.0140	**0.0008**	–	0.0039
$\|d_1 - d_2\|$ between ecotype	S	L	V3		0.0094	0.0058	–	0.0043
$\|d_1 - d_2\|$ between ecotype	S	L	V4		0.0089	0.0078	–	0.0047
$\|d_1 - d_2\|$ between ecotype	E	L	V6		0.0098	0.0153	–	0.0051
$\|d_1 - d_2\|$ between ecotype	E	S	V3		0.0091	0.0274	–	0.0057
$\|d_1 - d_2\|$ between ecotype	E	S	V6		0.0079	0.0408	–	0.0064
$\|d_1 - d_2\|$ between ecotype	E	S	V4		0.0064	0.0732	–	0.0073
$\|d_1 - d_2\|$ between ecotype	E	L	V1		0.0054	0.1489	–	0.0085
$\|d_1 - d_2\|$ between ecotype	E	L	V4		0.0025	0.4902	–	0.0102
$\|d_1 - d_2\|$ between ecotype	S	L	V6		0.0019	0.5651	–	0.0127
$\|d_1 - d_2\|$ between ecotype	S	L	V5		0.0017	0.5982	–	0.0170
$\|d_1 - d_2\|$ between ecotype	E	S	V2		0.0005	0.8974	–	0.0253
$\|d_1 - d_2\|$ between ecotype	E	L	V3		0.0003	0.9390	–	0.0500
|VCl within ecotype	E		V1	V2	0.8180	0.1010	–	0.0034
|VCl within ecotype	E		V5	V6	0.7905	0.1522	–	0.0037
|VCl within ecotype	E		V3	V5	0.7621	0.1842	–	0.0039
|VCl within ecotype	E		V1	V3	0.7187	0.2325	–	0.0043
|VCl within ecotype	E		V2	V3	0.6858	0.2749	–	0.0047
|VCl within ecotype	E		V2	V4	0.5976	0.3906	–	0.0051
|VCl within ecotype	E		V3	V6	0.6009	0.4345	–	0.0057
|VCl within ecotype	E		V2	V5	0.2198	0.5907	–	0.0064
|VCl within ecotype	E		V3	V4	0.1899	0.6358	–	0.0073
|VCl within ecotype	E		V4	V6	0.3991	0.6461	–	0.0085
|VCl within ecotype	E		V4	V5	0.2461	0.7903	–	0.0102
|VCl within ecotype	E		V1	V6	0.0748	0.8570	–	0.0127
|VCl within ecotype	E		V2	V6	0.1697	0.8584	–	0.0170
|VCl within ecotype	E		V1	V5	0.0975	0.9190	–	0.0253
|VCl within ecotype	E		V1	V4	0.0276	0.9779	–	0.0500
|VCl within ecotype	L		V5	V6	0.9519	**0.0009**	–	0.0034
|VCl within ecotype	L		V3	V6	0.9467	**0.0016**	–	0.0037
|VCl within ecotype	L		V3	V5	0.9269	**0.0039**	–	0.0039
|VCl within ecotype	L		V1	V4	0.6945	0.2558	–	0.0043
|VCl within ecotype	L		V1	V6	0.4261	0.2735	–	0.0047
|VCl within ecotype	L		V1	V5	0.5927	0.4086	–	0.0051
|VCl within ecotype	L		V1	V2	0.5952	0.4150	–	0.0057
|VCl within ecotype	L		V3	V4	0.3068	0.4437	–	0.0064
|VCl within ecotype	L		V4	V5	0.5239	0.5114	–	0.0073
|VCl within ecotype	L		V2	V5	0.2274	0.5717	–	0.0085
|VCl within ecotype	L		V2	V6	0.3186	0.7538	–	0.0102
|VCl within ecotype	L		V1	V3	0.2473	0.8166	–	0.0127
|VCl within ecotype	L		V4	V6	0.2377	0.8214	–	0.0170
|VCl within ecotype	L		V2	V4	0.1648	0.8833	–	0.0253
|VCl within ecotype	L		V2	V3	0.0037	0.9977	–	0.0500
|VCl within ecotype	S		V2	V5	0.7253	0.0232	–	0.0034
|VCl within ecotype	S		V5	V6	0.8098	0.0867	–	0.0037
|VCl within ecotype	S		V1	V5	0.8162	0.0896	–	0.0039
|VCl within ecotype	S		V1	V2	0.8034	0.0968	–	0.0043
|VCl within ecotype	S		V2	V6	0.7247	0.2083	–	0.0047
|VCl within ecotype	S		V1	V4	0.7206	0.2192	–	0.0051
|VCl within ecotype	S		V1	V6	0.4520	0.2292	–	0.0057

Appendix 2 Continued.

Test type	E 1	E 2	C 1	C 2	Observed value	$P(X_{Rand} \geq x)$	$P(X_{Rand} \leq x)$	α
IVCI within ecotype	S		V3	V6	0.6146	0.3727	–	0.0064
IVCI within ecotype	S		V3	V4	0.3275	0.4080	–	0.0073
IVCI within ecotype	S		V4	V5	0.5070	0.5366	–	0.0085
IVCI within ecotype	S		V3	V5	0.3391	0.7254	–	0.0102
IVCI within ecotype	S		V1	V3	0.2668	0.7938	–	0.0127
IVCI within ecotype	S		V2	V4	0.1660	0.8825	–	0.0170
IVCI within ecotype	S		V2	V3	0.0982	0.9291	–	0.0253
IVCI within ecotype	S		V4	V6	0.0952	0.9298	–	0.0500
IVCI between ecotypes	E	S	V6		0.8676	**0.0011**	–	0.0028
IVCI between ecotypes	S	L	V6		0.6963	0.0326	–	0.0030
IVCI between ecotypes	S	L	V5		0.6321	0.0575	–	0.0032
IVCI between ecotypes	E	S	V3		0.6012	0.0818	–	0.0034
IVCI between ecotypes	E	S	V5		0.6061	0.0822	–	0.0037
IVCI between ecotypes	S	L	V4		0.5591	0.1065	–	0.0039
IVCI between ecotypes	S	L	V3		0.5632	0.1068	–	0.0043
IVCI between ecotypes	E	L	V6		0.4717	0.1898	–	0.0047
IVCI between ecotypes	E	S	V2		0.4310	0.2593	–	0.0051
IVCI between ecotypes	E	S	V1		0.4103	0.2846	–	0.0057
IVCI between ecotypes	E	L	V3		0.3821	0.3010	–	0.0064
IVCI between ecotypes	E	L	V2		0.3021	0.4261	–	0.0073
IVCI between ecotypes	E	L	V1		0.2233	0.5585	–	0.0085
IVCI between ecotypes	E	S	V4		0.1898	0.6338	–	0.0102
IVCI between ecotypes	E	L	V4		0.1655	0.6662	–	0.0127
IVCI between ecotypes	S	L	V1		0.1503	0.6975	–	0.0170
IVCI between ecotypes	E	L	V5		0.0737	0.8495	–	0.0253
IVCI between ecotypes	S	L	V2		0.0260	0.9500	–	0.0500