J. evol. biol. 11 (1998) 465–480
1010–061X/98/040465–16 $ 1.50 + 0.20/0

Journal of Evolutionary Biology

Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail

T. J. DeWitt

Department of Biology, Binghamton University (SUNY), Binghamton, NY 13902-6000, USA
Current address: Center for Ecology, Evolution, & Behavior, TH Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40506-0225, USA, e-mail: DeWitt@ceeb.uky.edu

Key words: Costs of plasticity; evolution of plasticity; reaction norm; predator-induced defence; genetics of plasticity.

Abstract

Potential constraints on the evolution of phenotypic plasticity were tested using data from a previous study on predator-induced morphology and life history in the freshwater snail *Physa heterostropha*. The benefit of plasticity can be reduced if facultative development is associated with energetic costs, developmental instability, or an impaired developmental range. I examined plasticity in two traits for 29 families of *P. heterostropha* to see if it was associated with growth rate or fecundity, within-family phenotypic variance, or the potential to produce extreme phenotypes. Support was found for only one of the potential constraints. There was a strong negative selection gradient for growth rate associated with plasticity in shell shape ($\beta = -0.3$, $P < 0.0001$). This result was attributed to a genetic correlation between morphological plasticity and an antipredator behavior that restricts feeding. Thus, reduced growth associated with morphological plasticity may have had unmeasured fitness benefits. The growth reduction, therefore, is equivocal as a cost of plasticity. Using different fitness components (e.g., survival, fecundity, growth) to seek constraints on plasticity will yield different results in selection gradient analyses. Procedural and conceptual issues related to tests for costs and limits of plasticity are discussed, such as whether constraints on plasticity will be evolutionarily ephemeral and difficult to detect in nature.

Introduction

Natural environments are inherently dynamic such that no single phenotype is consistently optimal (Ford, 1964; Levins, 1968; Hassell, 1978). Natural selection, therefore, will favor organisms that are capable of altering their development to track environmental changes (Schmalhausen, 1949; Bradshaw, 1965). For example, many empirical studies show that predators trigger the production of defensive phenotypes in prey, implying that phenotypic plasticity is an adaptation (reviewed by Harvell, 1990; Karban and Baldwin, 1997). With this example, the benefit of plasticity relative to fixed, undefended phenotypes is obvious; protection from predators is gained when predators occur. When predators are absent, plasticity can save the cost of producing and maintaining costly defenses that are not needed. Because plasticity can offer advantages in both the inducing and noninducing environments, it superficially can seem to be an unbeatable strategy.

Although the benefits of phenotypic plasticity are clear, the costs and limits that constrain its evolution are more obscure. *Costs* of plasticity are defined as fitness deficits associated with plastic genotypes relative to fixed genotypes producing the same mean phenotype in a focal environment (DeWitt et al., 1998; Scheiner and Berrigan, 1998). *Limits* of plasticity are functional constraints that reduce the benefit of plasticity compared to perfect plasticity, which is the production of perfect phenotype-environment matching in all instances. Costs of plasticity, in particular, are consistently mentioned in conceptual reviews about the evolutionary and ecological importance of plasticity (e.g., Schlichting, 1986; Futuyma and Moreno, 1988; Newman, 1992; Sultan, 1992; Scheiner, 1993; Via et al., 1995; DeWitt et al., 1998) and theoretical models have explored the consequences of costs for the evolution of plasticity (Van Tienderen, 1991; Leon, 1993). Despite the conceptual attention, surprisingly little empirical work has explicitly tested for costs of plasticity. Van Tienderen (1991) has even proposed a method to test for costs, yet no empirical studies have used this approach. As well, little empirical work has been published on limits of plasticity, but models of these limits are common (e.g., Cohen, 1967; Lively, 1986; Moran, 1992; Leon, 1993; Getty, 1996; Padilla and Adolph, 1996). Empirical work is badly needed in this field because costs (and limits) are probably the least-understood components of the evolution of plasticity (Newman, 1992; Via et al., 1995).

Costs and limits of plasticity

A recent review (DeWitt et al., 1998) distinguished five costs and four limits of plasticity. Costs of plasticity result from: (C1) *maintenance costs* of the sensory and regulatory mechanisms that produce plasticity; (C2) *production costs* of inducible phenotypes in excess of costs payed by fixed genotypes to produce the same phenotype; (C3) *information acquisition costs* obtained during environmental sampling; (C4) *developmental instability costs* that result if plastic development is more variable than fixed development; and (C5) *genetic costs* such as linkage of plasticity

genes with genes conferring low fitness. Limits of plasticity include: (L1) *information reliability limits* associated with imperfect correlations between the cue that triggers plasticity and the true state of the environment; (L2) *lag time limits* where there is a delay in sensing and responding to environmental information; (L3) *developmental range limits* which occurs if plastic development is incapable of producing extreme phenotypes that are possible through fixed development; and (L4) an *epiphenotype problem*, where add-on phenotypes may be less effective than developing the phenotype during early ontogeny. Many of the constraints have been described adequately in the literature, with the exceptions being production costs (C2), developmental instability costs (C4), developmental range limits (L3), and the epiphenotype problem (L4).

Character production costs have been demonstrated for several inducible traits (e.g., Stemberger, 1988; Black and Dodson, 1990; plant literature reviewed by Sultan, 1992). Production costs affect the net adaptive value of plasticity, but they are not necessarily costs of plasticity. In a noninducing environment, production costs measure the advantage of plasticity over fixed expression of a costly character state; production costs are what facultative trait expression saves. Likewise, production costs measure the disadvantage for plastic genotypes which mistakenly produce an unnecessary phenotype. In this way, character production costs determine the potential magnitude of information reliability problems (L1). Production costs should not be considered costs of plasticity when genotypes that obligately produce a phenotype and those that produce the trait facultatively are likely to incur the same energetic and material debt to express the trait (Newman, 1992; DeWitt, 1996). Only the portion of production costs unique to plastic development of a given phenotype should be considered costs of plasticity (Scheiner and Berrigan, 1998). For example, fixed development can integrate character production into ontogeny when costs are low, whereas facultative development is contingent upon environmental rather than economic circumstances (Scheiner, personal communication). Similarly, production of structures based on environmental cues implies that inducible characters often must be added to an existing phenotype. It may be impossible to create a post-hoc phenotypic addition as effectively as if it were integrated with early development (''the epiphenotype problem''; DeWitt et al., 1998).

The facultative nature of plastic development requires that a genome encode accessory developmental pathways and associated buffering systems, sensory systems to guide development, and perhaps other developmental components. The complex nature of the trait logically implies two potential problems. First, plastic development might be intrinsically unstable, which could manifest as large variance (''developmental noise'') associated with phenotype means (DeWitt, 1996). Variance around phenotype means can be costly under stabilizing selection because noisy development cannot precisely match phenotypic optima (Yoshimura and Shields, 1995). A link between developmental noise and plasticity, therefore, could represent a cost of plasticity (DeWitt, 1996). Second, plastic development, by maintaining extra ''developmental baggage'', may be unable to produce extreme phenotypes (Wilson and Yoshimura, 1994). This effect could manifest within a focal environment as a negative correlation between plasticity and phenotype means (DeWitt, 1996). An example of such an effect is illustrated in Figure 1.

The purpose of the present work is to test for energetic costs (C1, C2, C3, and C5), developmental instability costs (C4), and developmental range limits (L3) associated with plasticity. Hypothesis tests were performed with data derived from an empirical study on predator-induced morphology and life history in the freshwater pulmonate snail, *Physa heterostropha* raised with pumpkinseed sunfish (*Lepomis gibbosus*), with crayfish (*Orconectes obscurus*), or without predators (DeWitt, 1996). The functional significance of these traits makes them ideal to seek costs, because costly plasticity will be evolutionarily ephemeral if no benefits exist to compensate the costs (DeWitt et al., 1998).

Methods

Study system

The freshwater snail *Physa* (=*Physella*) occurs in a diversity of habitats worldwide and is often common in stressful (e.g., stochastic) environments that exclude other species (Burch, 1989; Wethington and Dillon, 1991). In eastern North America, physid snails are susceptible to many predators, particularly crayfish and molluscivorous sunfish (Snyder, 1967; Osenberg and Mittelbach, 1989). The *Physa*-crayfish-fish system is ideal for plasticity studies because the predators differ fundamentally in how they locate, choose, and ingest prey. Snails that alter their phenotype to reduce predation by fish increase their vulnerability to crayfish and vice versa (DeWitt, 1996). Fish and crayfish exert selection pressure on both shell shape and size. Molluscivorous sunfish are shell-crushing predators. As such, they are deterred by rotund shell morphology because rotund shells are hard to crush (DeWitt, 1996). Crayfish are shell-entry predators that are deterred by elongate

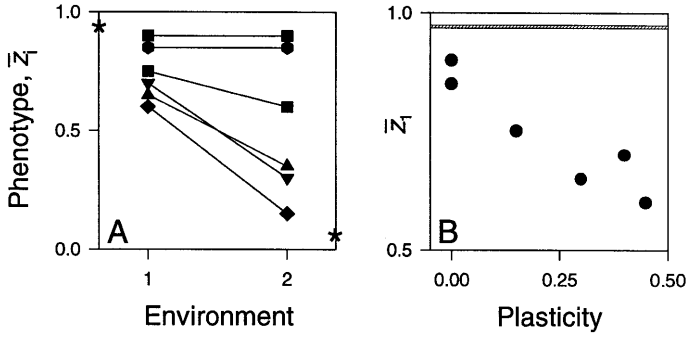

Fig. 1. A developmental specialist-generalist tradeoff. Fixed genotypes (developmental specialists) are capable of producing more extreme phenotypes than plastic genotypes (developmental generalists). A. Reaction norms illustrating one of several possible manifestations of the tradeoff. Asterisks indicate the end of the phenotype distribution that is most adaptive for each environment. B. The correlation from data in Panel A between the phenotype expressed in Environment 1 and the amount of plasticity across environments. The rule line indicates the optimum phenotype for the environment. Genotypes with greater plasticity lie farther from the optimum phenotype.

shell morphology, because elongate physid shells have narrow apertures that are difficult to reach into (DeWitt, 1996). For snail size, the focal fish in this study (pumpkinseed sunfish, *Lepomis gibbosus*) prefer relatively large snails (roughly >7 mm; Osenberg and Mittelbach, 1989). Crayfish differentially eat small snails (<7 mm; Crowl and Covich, 1990).

Physid snails mitigate the functional tradeoffs between predators with adaptive phenotypic plasticity: Snails raised with fish exhibit rotund shells and slow growth compared with their sibs raised with crayfish, which exhibit elongate shells and fast growth (DeWitt, 1996). There was variation in reaction norms for both traits (i.e., $P \leq 0.05$), but the magnitude of this variance component was low for shell shape relative to the magnitude of genetic and environmental main effects. The primary report of the rearing experiment is in DeWitt (1996). Here those data are used to test for costs and limits of plasticity.

Experimental design and estimation of plasticity

DeWitt's (1996) study addressed plasticity in shell shape, growth rate, fecundity, and behavior of 29 families of *P. heterostropha* raised in water without predators, water with predatory pumpkinseed sunfish, and water with predatory crayfish (*Orconectes obscurus*). One hundred snails were collected from the Susquehanna River in Binghamton, New York, brought to the lab, and held in isolation of one another for a week in conditioned tap water with ad libitum food. Egg masses layed in the first three days of the following week were used in the study ($N = 33$ families initially). Since these animals were collected from high density populations, they would have been using stored sperm to fertilize eggs, primarily from the last sperm donor (Wethington and Dillon, 1991). Thus, sibs were likely to be related by 0.5 (full-sibs) but could have been related by slightly less. Mortality was negligible in the study, but some individuals escaped from their rearing containers, resulting in four families being removed from the study. Predators were fed nonexperimental snails. Experimental snails were fed spirulina flakes for the duration of the experiment, which ran until 51–54 days after the eggs hatched.

At the end of the experiment, I assayed snail behavior in each environment by noting the number of families with members that had crawled out of the water. I measured family mean fecundity in each environment as the total number of egg masses produced during the course of the experiment. Morphology of all individuals was measured as aspect ratio (shell length divided by width). Growth rate was measured as the increase in the shell profile (in mm^2/d). Shell shape and growth rate were not genetically correlated. Further details about the measurement techniques and rearing conditions are in DeWitt (1996).

Following Schlichting (1986), the degree of plasticity for a trait, z, was obtained as the difference in family mean phenotype between environments. With three categorical environments, there are three environmental pairs for which I could define plasticity:

$$P_{F\text{-}C} = |\bar{z}_F - \bar{z}_C| \quad (1)$$

$$P_{W\text{-}F} = |\bar{z}_W - \bar{z}_F| \quad (2)$$

$$P_{W\text{-}C} = |\bar{z}_W - \bar{z}_C|\,, \quad (3)$$

where $\bar{z}$ is the family mean phenotype for the environment denoted in subscript (F = fish; C = crayfish; W = water). Absolute values were used so the difference would indicate plasticity per se (slope in the reaction norm), regardless of direction.

To limit the number of statistical tests, the plasticities used to seek costs (Eq. 1, 2 or 3) were determined by the functional ecology of the system. Specifically, there is no need to test for costs unless plasticity is known or suspected to mitigate a functional tradeoff (DeWitt et al., 1998). For shell shape, there was no evidence of character production costs, so there should be no energetic savings available through facultative trait expression that would indicate a tradeoff between predatory and nonpredatory environments. A morphological tradeoff does exist between the fish and crayfish environments because defense against one predator is at the expense of defense against the other. Thus, I used $P_{\text{F-C}}$ to test for energetic (growth and fecundity) costs of morphological plasticity.

I used $P_{W\text{-}F}$ and $P_{W\text{-}C}$ to test for fecundity costs of growth rate plasticity. There are two reasons to use the separate measures for growth plasticity. First, growth often exhibits a negative genetic correlation with fecundity because both processes draw on finite energetic reserves (Roff, 1992; Stearns, 1992). Thus, a character production cost is unavoidable because energy diverted to growth reduces the potential investment in fecundity. Second, growth plasticity is likely to result from different mechanisms for the two predators. Reduced growth in response to fish was attributed to antipredator behavior (DeWitt, 1996); whereas accelerated growth in response to crayfish results from egg reservation (Crowl and Covich, 1990; DeWitt, 1996).

Tests for energetic costs of plasticity (C1, C2, C3, C5)

Van Tienderen (1991) suggested the following statistical model to assess costs of plasticity using quantitative genetic data:

$$W_{i,j,k=1} = \text{Constant} + (\beta_1 * z_{i,j,k=1}) + (\beta_2 * \bar{z}_{j,k=2})\,, \quad (4)$$

where $W_{i,j,k}$ is a fitness component for individual i of family j in environment $k = 1$. The term $\bar{z}_{j,k}$ is the mean phenotype of family i in an alternative environment ($k = 2$). Note that the model is conceptually analogous if $\bar{z}_{j,k=2}$ is replaced with P_j (because $P_j = \bar{z}_{j,k=2} - \bar{z}_{j,k=1}$ or the absolute value of the difference). The analysis is performed for each environment.

Van Tienderen's method is a selection gradient analysis (a regression of trait values on fitness components; Lande and Arnold, 1983). Betas (fitted partial regression coefficients) estimate selection coefficients describing how the trait value in a focal environment affects fitness (β_1) and how the ability to be plastic manifests

as fitness effects (β_2), even though plasticity, by definition, is not expressed by an individual. This method simultaneously tests and controls for simple character production costs. It also controls for the fact that some environments are more stressful than others (i.e., environment costs), because the test is performed within environments. Inspection of residuals from the model can be used to determine whether quadratic terms are needed to estimate stabilizing selection or whether an alternative method of defining plasticity would be useful. I used Van Tienderen's method to test for growth rate costs of morphological plasticity in shell shape.

I used family-mean models to test for fecundity reductions associated with morphological and growth-rate plasticity, where:

$$W_{j,k=1} = \text{Constant} + (\beta_1 * \bar{z}_{j,k=1}) + (\beta_2 * P_j)\,. \tag{5}$$

This model was used for tests involving fecundity, because I measured total family fecundity rather than individual egg production. The family means model is analogous to Van Tienderen's individual model but is less powerful. Specifically, if deviations of individual phenotypes from their family mean can not be used to explain variance, the magnitude of error variance will be that much greater relative to the plasticity variance component.

Costs of plasticity should manifest as a significant negative selection coefficient for the plasticity term in these models. However, such a result would not indicate which underlying mechanism produced the cost (C1, C2, C3, C4, or C5). A specific test for production costs (C2) was suggested by Scheiner and Berrigan (1998): Adding an interaction term to Van Tienderen's model could test specifically whether character production costs are greater for more plastic genotypes (indicated by a significant β for the interaction term $z \times P$). When costs of plasticity were suggested in my primary analyses, I tested for this interaction term.

Tests for developmental instability (C4)

Developmental instability can be measured as the within-environment variance for a family in a given environment (Scheiner et al., 1991). Two potential problems can arise with this method: (i) there may be mean-variance correlations which can affect the interpretation of results, and (ii) unbalanced experimental designs may lead to families with fewer individuals having a greater variance estimate due to sampling error, rather than to developmental instability per se. My design was not badly imbalanced (mean family size within each environment was 8.60 with a standard error of 0.21) nor were there significant mean-variance correlations (for shell aspect ratio, $r \leq 0.30$, $\text{df} = 29$, $P > 0.1$ for each environment; for growth rate, $r \leq 0.11$, $\text{df} = 29$, $P > 0.5$). Nevertheless, I employed safeguards against these problems at the expense of two degrees of freedom in the analysis by using the family size and trait mean as covariates. Another approach to the mean-variance problem would be to use the coefficient of variation rather than variance. Using the mean as a covariate is a more flexible approach because it controls for many forms of

mean-variance relationships in addition to that explained by simply dividing the square root of variance by the mean. I used regression analysis to partition trait variance in a focal environment into family size, family mean, and plasticity effects. A significantly positive partial regression coefficient for a plasticity term would support the developmental instability hypothesis.

Tests for developmental range limit (L3)

The developmental range hypothesis makes the prediction that the most extremely specialized phenotypes in each environment will be produced by the least plastic genotypes. I used correlation analysis to test for an association between the family mean in a focal environment and the degree of plasticity across environments. Negative correlations in the crayfish environment would indicate that extreme trait values (high growth or aspect ratio) were achieved by families with low plasticity, and would support the idea that plasticity limits developmental range. Conversely, the developmental range limit hypothesis would be supported by positive correlations in the fish environment.

Results

Energetic costs (C1, C2, C3, C5)

There was no indication of character production costs in any of the analyses, indicating that rotund and elongate shells were equally costly or cost-free (Tab. 1). Likewise, with one exception, there was little indication that members of high-plasticity families suffered reduced growth or fecundity compared to members of low-plasticity families (Tab. 1). The exception was that members of families with high plasticity in shell shape grew poorly in the fish environment (Tab. 1A). The growth selection gradient against plasticity was 0.30 (0.29 if nonsignificant factors were removed from the model; $t = 4.76$, $P < 10^{-5}$). This relationship is plotted in Figure 2, using residual growth from a regression of the trait value and family size on growth. For clarity, data were grouped by family for presentation in the figure to reduce the number of points (from 251 to 29) and to provide visual reference as to the magnitudes of within- and between-family variances. The growth deficit in the fish environment associated with morphological plasticity led me to test for an interaction between the plasticity and trait mean terms as suggested by Scheiner and Berrigan (1998). The interaction was not significant ($t = 0.82$, $P = 0.4$), indicating that there was no added character production cost associated with plastic development. Inspection of residuals from the selection gradient analyses of Table 1 did not suggest that there was stabilizing selection for plasticity.

Developmental instability (C4)

Relatively plastic families did not exhibit greater within-environment variation for either aspect ratio or growth (for all tests, $P > 0.3$; Tab. 2). Thus, develop-

Table 1. Regression analyses testing for energetic costs of plasticity (C1, C2, C3, and C5). Family size and aspect ratio terms are control measures to facilitate better tests of the plasticity term, which provides the hypothesis test (shown in bold). The form of plasticity used to seek costs (Eq. 1, 2, or 3 from the text) is indicated as subscript, where F = fish, C = crayfish, and W = predator-free water.

Environment	Source of variation	Standardized regression coefficient	Model			
			t	P	R^2	N
A. Growth rate costs of morphological plasticity						
No-predator	Family size	−0.459	−8.01	$<10^{-6}$	0.22	243
	Aspect ratio	0.056	0.98	0.328		
	Plasticity $_{F\text{-}C}$	**−0.037**	**−0.64**	**0.522**		
Fish	Family size	−0.097	−1.60	0.111	0.10	251
	Aspect ratio	−0.110	−1.79	0.074		
	Plasticity$_{F\text{-}C}$	**−0.297**	**−4.83**	**$<10^{-5}$**		
Crayfish	Family size	−0.325	−5.42	$<10^{-6}$	0.13	253
	Aspect ratio	0.089	1.43	0.155		
	Plasticity$_{F\text{-}C}$	**0.042**	**0.66**	**0.509**		
B. Fecundity costs of morphological plasticity						
No-predator	Family size	0.016	0.08	0.938	0.01	29
	Aspect ratio	0.022	0.11	0.328		
	Plasticity$_{F\text{-}C}$	**−0.065**	**−0.32**	**0.522**		
Fish	Family size	−0.263	−1.46	0.158	0.19	29
	Aspect ratio	0.140	0.72	0.481		
	Plasticity$_{F\text{-}C}$	**−0.278**	**−1.42**	**0.167**		
Crayfish	Family size	−0.547	−3.40	0.002	0.36	29
	Aspect ratio	0.242	1.22	0.235		
	Plasticity$_{F\text{-}C}$	**−0.151**	**−0.76**	**0.454**		
C. Fecundity costs of growth rate plasticity						
No-predator	Family size	0.487	1.25	0.224	0.29	29
	Growth rate	0.974	2.67	0.013		
	Plasticity$_{W\text{-}F}$	**−0.387**	**−1.39**	**0.179**		
	Plasticity$_{W\text{-}C}$	**−0.122**	**−0.57**	**0.576**		
Fish	Family size	−0.177	−1.16	0.259	0.45	29
	Growth rate	0.649	3.37	0.003		
	Plasticity$_{W\text{-}F}$	**0.171**	**0.94**	**0.355**		
	Plasticity$_{W\text{-}C}$	**0.028**	**0.15**	**0.883**		
Crayfish	Family size	−0.625	−2.45	0.022	0.35	29
	Growth rate	−0.156	−0.63	0.537		
	Plasticity$_{W\text{-}F}$	**−0.030**	**−0.17**	**0.866**		
	Plasticity$_{W\text{-}C}$	**0.152**	**0.83**	**0.416**		

mental instability does not appear to be a consequence of phenotypic plasticity in shell shape and growth rate for this sample of snails.

Developmental range limit (L3)

Members of high-plasticity families did not exhibit a limited developmental range (Tab. 3). The most plastic families produced the most rotund shells in the fish environment and the most elongate shells in the crayfish environment. Similarly, the slowest growth in the fish environment was exhibited by the most plastic families. Each of these trends opposed my predictions based on limits to plasticity.

Discussion

I detected only one line of evidence for costs or limits of plasticity. Plasticity of shell morphology was associated with reduced growth in the fish environment (Fig. 2). This result is perhaps the first evidence for a cost of plasticity, yet the analysis

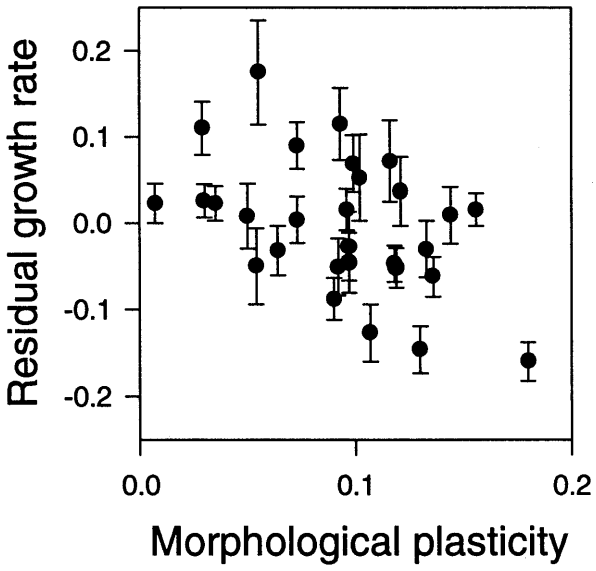

Fig. 2. Growth reduction in the fish environment associated with morphological plasticity across predatory environments. Residual growth rates were obtained from a regression of individual phenotypes and rearing densities on growth rate. Data were grouped by family for clarity. Using residual values statistically controls for character production costs (Van Tienderen, 1991), to reveal selection gradients associated with plasticity per se.

Table 2. Tests for association between developmental variance and plasticity (C4). Details are as in Table 1.

Environment	Source of variation	Standardized regression coefficient	Model			
			t	P	R^2	N
A. Tests with morphological plasticity						
No-predator	Family size	−0.052	−0.27	0.790	0.10	29
	Aspect ratio	0.299	1.56	0.131		
	Plasticity$_{F\text{-}C}$	**−0.006**	**−0.03**	**0.977**		
Fish	Family size	0.062	0.33	0.746	0.11	29
	Aspect ratio	0.351	1.71	0.100		
	Plasticity$_{F\text{-}C}$	**0.134**	**0.66**	**0.518**		
Crayfish	Family size	−0.286	−1.54	0.137	0.14	29
	Aspect ratio	0.023	0.10	0.923		
	Plasticity$_{F\text{-}C}$	**0.237**	**1.03**	**0.312**		
B. Tests with growth rate plasticity						
No-predator	Family size	−0.349	−0.78	0.441	0.07	29
	Growth rate	0.070	0.17	0.869		
	Plasticity$_{W\text{-}F}$	**−0.215**	**−0.67**	**0.507**		
	Plasticity$_{W\text{-}C}$	**−0.217**	**−0.88**	**0.385**		
Fish	Family size	0.151	0.93	0.360	0.39	29
	Growth rate	0.609	3.00	0.006		
	Plasticity$_{W\text{-}F}$	**0.277**	**1.45**	**0.160**		
	Plasticity$_{W\text{-}C}$	**0.075**	**0.38**	**0.709**		
Crayfish	Family size	0.302	1.00	0.325	0.10	29
	Growth rate	−0.005	−0.02	0.986		
	Plasticity$_{W\text{-}F}$	**−0.065**	**−0.31**	**0.758**		
	Plasticity$_{W\text{-}C}$	**0.023**	**0.11**	**0.916**		

Table 3. Tests for developmental range limits (L3). The expected sign if plasticity were to limit developmental range is given in column 5.

Trait 1	Trait 2	Correlation	P	Expected sign
Aspect ratio plasticity$_{F\text{-}C}$	Aspect ratio$_F$	−0.389	0.037	+
	Aspect ratio$_C$	0.588	0.001	−
Growth plasticity$_{W\text{-}F}$	Growth rate$_F$	−0.324	0.087	+
Growth plasticity$_{W\text{-}C}$	Growth rate$_C$	0.248	0.195	−

Note: Correlations were performed using the 29 family means.

by itself does not reveal the root cause of the cost. We can exclude only one possibility, production costs of plasticity, because there was no significant interaction between character values and plasticity (sensu Scheiner and Berrigan, 1998). It often will be difficult to determine the specific mechanisms underlying costs or limits because plasticity, like any other trait, can involve many component developmental or regulatory processes that are correlated with one another either genetically or through mechanical/functional constraints (Arnold, 1994). In the present study, however, a possible explanation can be inferred from the functional ecology and quantitative genetics of the system. Snail families exhibiting strong induced morphological responses to predators tended to have strong induced growth responses. For example, the genetic correlation between $P_{F\text{-}C}$ for growth and aspect ratio was $r_G = 0.49$ ($df = 29$, $P = 0.007$). Reduced growth in the presence of fish was attributed by DeWitt (1996) to an antipredator behavior. Over 70% of families had individuals that crawled out of the water in response to fish, compared to 10% of families with members responding to crayfish (DeWitt, 1996). Snails do not feed while out of the water, so the response is likely to decrease growth.

The cause of the correlation between morphological and growth plasticity is unknown, but could result from several mechanisms. For example, both plasticities may rely on the same information acquiring system (e.g., chemoreceptors) to inform development about the state of the environment. Such a mechanism could generate the genetic correlation between morphological plasticity and reduced growth in the fish environment. If so, should the growth reduction associated with morphological plasticity be considered a cost of plasticity? Stearns (1989) argued that genetic correlations of plasticity with other traits that are costly are costs of plasticity. The present study is the second to suggest a genetic correlation between plasticity in one trait and a cost related to another trait. Newman (1988) examined five family reaction norms of spadefoot toad development time and metamorph size in response to pond duration, which was either long or short. The family exhibiting the greatest plasticity in metamorph size (Family 5) had the longest development time. Extended development was considered to be a cost for Family 5's plasticity. Extended development may indeed be costly, because it increases dessication risk (Newman, 1992), but it may reflect a character production cost. That is, even a fixed genotype producing a large body size would presumeably require extended development time to do so. Thus, we can not use Newman's data to distinguish a cost of plasticity from character production costs. Van Tienderen's (1991) method statistically controls for production costs, so that costs due to plasticity are not confounded with production costs.

There was no evidence of character production costs in the present study. However, two conceptual problems should be addressed. First, the behavior that is thought to reduce growth (crawling out of the water) may have a fitness benefit, reduced predation, that outweighs the cost to growth. Using alternative fitness components could yield different results in tests for costs of plasticity. From this perspective, the growth reduction documented in the present study is equivocal as a cost of plasticity. That is, it can be considered as a cost, but perhaps it is not a net cost of plasticity. Second, the distinction between various costs and limits becomes blurred in instances such as this. Regardless of whether the growth

reduction associated with morphological plasticity is a net cost of plasticity, the data provide an estimate of the severity of errors associated with incorrect information about the state of the environment (L1). If mistaken responses to environmental cues reduce growth, then we know how important the reliability of environmental cue is in the evolution of plasticity. It is important that tests for costs and limits of plasticity be conducted with knowledge of functional ecology, to best understand what factors determine the evolution of plasticity. A multivariate approach to the evolution of plasticity is essential (see also Spitze and Sadler, 1996).

I detected no evidence of the other constraints for which I tested: Developmental instability (C4) or developmental range limits (L3) associated with plasticity. Several other studies have measured both developmental stability and plasticity, but they failed to yield consistent results. Instead, associations between the two developmental parameters tend to be trait- or environment-specific trends (reviewed by Scheiner et al., 1991; see also Yampolsky and Scheiner, 1994). In perhaps the largest study, Scheiner et al. (1991) raised more than 50 000 *Drosophila melanogaster* to examine plasticity in wing length, thorax length, and sternopleural bristle number in two thermal environments (19 and 25 °C), while also measuring developmental instability. They measured developmental instability both as the within-environment variance and as fluctuating asymmetry. They found no general relationship between plasticity and developmental instability. For example, within-environment variance for bristle number was higher for more plastic families, but only at 25 °C. Since this was also the environment that induced greater bristle numbers, the result could even reflect a mean-variance correlation. Mean-variance correlations, or correlations between the mean and fluctuating asymmetry, are probably common. For example, in another fly (the midge *Chironomus plumosus*), larger individuals exhibited the most fluctuating asymmetry in wing length (McLachlan and Cant, 1995). The clearest evidence for correlations between plasticity and developmental instability will occur either when the most plastic genotypes have greater developmental instability in both environments, or, when measures are taken to statistically control for mean-variance correlations in tests for plasticity-variance associations.

Selection experiments have also examined the link between plasticity and developmental noise. Many researchers have selected for increased or decreased plasticity and found no change in developmental variance (reviewed in Scheiner et al., 1991). In one study (Waddington and Robertson, 1966), selection for increased plasticity increased developmental variance. A problem here is that directional selection causes homozygosity, which in turn can produce developmental instability (Thoday, 1955; Palmer and Strobeck, 1986). The strongest demonstration of a plasticity-instability connection requires that selection for increased plasticity increases developmental instability while selection for decreased plasticity decreases instability. What we can conclude from the existing studies is that there is no necessary connection between developmental instability and plasticity.

The ecological and evolutionary implications of variation in plasticity have been widely discussed, often in the context of specialization and generalization (Lively, 1986; Van Tienderen, 1991; Wilson and Yoshimura, 1992; Spitz and

Sadler, 1996). Applying the concept of specialists and generalists to development, we might expect that developmental generalists (plastic genotypes) are unable to produce as extreme a phenotype as is required by extreme environments. I found the opposite; the most extreme phenotypes were produced by the most plastic families. Thus, I could not identify fixed-development families that could be called ''fish specialists'' or ''crayfish specialists''. Families with relatively fixed development produced intermediate phenotypes. These families could be called developmental specialists (they had shallow reaction norms) but phenotypic generalists (they produced intermediate phenotypes in all environments). Given the large number of existing studies on plasticity, it may be possible to reexamine published work for the existence of pure specialists with fixed development of extreme phenotypes.

Although little evidence yet exists for costs and limits of plasticity, the tests performed in this study were important to do because they involved functionally important forms of plasticity. For example, if plasticity were costly but of no functional importance, it would be evolutionarily ephemeral. It therefore only makes sense to seek constraints for adaptive forms of plasticity, because they can persist even when costly (DeWitt et al., 1998). The need to seek costs using adaptive plasticity poses a quandary, however, because adaptive plasticity may progress to fixation within populations. Variation in reaction norms is necessary to detect a cost of plasticity. Without variance in reaction norms, then no comparative basis exists to show that more plastic genotypes differ from less plastic genotypes. Although snail families in the present study differed enough in morphological plasticity to detect a negative association between plasticity and growth, there was low variance in plasticity (''$G \times E$ variance'') relative to the magnitude of variance due to genetic and environmental main effects (DeWitt, 1996). This pattern is common in studies of plasticity (Scheiner, 1993) and is perhaps the most difficult problem in tests for costs of plasticity. Even when plasticity is fixed for local optima (no variance within populations), there may still be variation in plasticity among populations, suggesting the importance of population-level comparisons.

One recent study tested for costs of plasticity using organisms from multiple populations. Scheiner and Berrigan (1998) examined plasticity of three functionally important traits (juvenile body length, body depth, and tail-spine length) in 47 distinct clones of *Daphnia pulex* from 22 populations. Using methods similar to those in the present paper, they found no significant energetic costs of plasticity for the two traits exhibiting variance in plasticity. More research is necessary before we can make generalizations regarding the existence and frequency of the various constraints on plasticity. It appears at present as if such constraints are neither universal nor great in magnitude. Costs and limits of plasticity may only be present for some plastic traits and for some organisms or populations. Also, as in the present study, cost or limits may only be evident in a subset of possible environments. One reason to expect a sporadic distribution of these constraints is that alleles conferring limited or costly plasticity are expected to be under selection for efficiency, so they should be replaced by alleles that confer plasticity less

expensively or with fewer limitations. In this way, costs and limits of plasticity should wane through evolutionary time. We may examine many case studies before detecting unqualified costs or limits, if indeed they exist. Reexamining data from past studies will be a profitable way to begin. In many cases the data needed to use Van Tienderen's (1991) method or the other methods I discussed already have been collected.

Acknowledgements

I thank D. S. Wilson, A. Sih, S. M. Scheiner, C. P. Grill, H. H. Wilkinson, B. D. Wisenden, and an anonymous reviewer for valuable comments on drafts of this paper. I am particularly grateful to Sam Scheiner for many lively and productive debates on the issues. This project was funded by the Pocono Comparative Lakes Program, Sigma Xi, and the Department of Biology of Binghamton University.

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Received 27 May 1997;
accepted 5 June 1997.