Phenotypic Plasticity, Costs of Phenotypes, and Costs of Plasticity

Toward an Integrative View

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Why are some traits constitutive and others inducible? The term *costs* often appears in work addressing this issue but may be ambiguously defined. This review distinguishes two conceptually distinct types of costs: phenotypic costs and plasticity costs. Phenotypic costs are assessed from patterns of covariation, typically between a focal trait and a separate trait relevant to fitness. Plasticity costs, separable from phenotypic costs, are gauged by comparing the fitness of genotypes with equivalent phenotypes within two environments but differing in plasticity and fitness. Subtleties associated with both types of costs are illustrated by a body of work addressing predator-induced plasticity. Such subtleties, and potential interplay between the two types of costs, have also been addressed, often in studies involving genetic model organisms. In some instances, investigators have pinpointed the mechanistic basis of plasticity. In this vein, microbial work is especially illuminating and has three additional strengths. First, information about the machinery underlying plasticity—such as structural and regulatory genes, sensory proteins, and biochemical pathways—helps link population-level studies with underlying physiological and genetic mechanisms. Second, microbial studies involve many generations, large populations, and replication. Finally, empirical estimation of key parameters (e.g., mutation rates) is tractable. Together, these allow for rigorous investigation of gene interactions, drift, mutation, and selection—all potential factors influencing the maintenance or loss of inducible traits along with phenotypic and plasticity costs. Messages emerging from microbial work can guide future efforts to understand the evolution of plastic traits in diverse organisms.

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In this age of the genome, phenotypic traits such as behavior, morphology, and physiology remain compelling to many researchers. Ecologists, for example, are interested in connecting variation in organismal traits with community and ecosystem patterns and processes (Eviner 2004; Miner *et al.* 2005). Developmental and molecular geneticists also examine variation in organismal traits, connecting this variation with underlying genetic mechanisms and biochemical pathways. Ecological geneticists and other evolutionary biologists are also interested in connecting phenotypes and associated genes, as well as in how both phenotypes and genotypes are altered by multiple evolutionary processes—such as natural selection, migration and gene flow, functional tradeoffs among multiple traits, pleiotropy, mutation, and genetic

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drift (Pigliucci 2001; Lee 2002; Schlichting & Smith 2002; West-Eberhard 2003; Weinig & Schmitt 2004; Pigliucci *et al.* 2006; Ghalambor *et al.* 2007; Masel *et al.* 2007).

To examine the interplay among phenotypic traits, genes, and such processes, a primary task for most phenotypic research is simply to examine the scope and pattern of trait variation. This critical task is often complicated by the phenomenon of phenotypic plasticity: variation in environmental conditions eliciting variation in the traits expressed by a given genotype. Conceptually, studying the phenotypic plasticity of traits requires recognizing an organism as a duality, as both a phenotype and a genotype. Doing so often involves the concept of a reaction norm: a genotype's range of phenotypes expressed as a function of the environment (Sarkar 1999; Pigliucci 2001). Operationally, studying plastic traits and reaction norms requires a biologist to meet a series of challenges. First, one must identify and specify groups of individuals with similar genotypes—clones, half-sibs, artificial selection lines, conspecifics. Then, one must characterize the phenotypes of these "replicate genotypes" when grown in two or more environments. Next, one must decide whether to focus not only on the different traits expressed in those environments but perhaps also on the traits' plasticities. That is, plasticity can be conceptualized as a complex trait in and of itself. Finally, one must decide whether one needs to obtain information about underlying physiological and genetic mechanisms regulating traits and their plasticity (Schlichting 1986; Via *et al.* 1995; Pigliucci 1996).

Woltereck, studying genetically homogeneous lines of the small crustacean *Daphnia* during the early 20th century, was among the first to grapple with the challenge of genotype–phenotype mapping. He documented the effects of many different environmental factors on variation in head height, a continuously varying quantitative trait of *Daphnia*'s exoskeleton (Sarkar 1999). He is credited with coining the term *Reaktionsnorm* (Schlichting & Pigliucci 1998), an idea that languished for decades before interest in phenotypic plasticity was rekindled during the mid-1960s and 1970s. Since then, the concept of the reaction norm has often been used alongside other ecological and quantitative genetic techniques. It has been a helpful unifying concept for empiricists and theoreticians studying traits that exhibit phenotypic plasticity (Via & Lande 1985; Endler 1986; De Jong 1995; Rose & Lauder 1996).

Often, research in phenotypic plasticity has progressed by zooming out, ignoring the details of the genes underlying a reaction norm. Instead, such work typically focuses on a particular plastic trait or traits, examining how selection acts on them or investigating their effect on ecological performance. With this perspective, many decisions must be made in translating the phenomenon of phenotypic plasticity into quantifiable terms. Often, models treat the plasticity of a trait as itself a complex, quantifiable trait. Then, variation in traits and in the plasticities of the traits can be conceptualized into discrete, hierarchical categories. The first issue is whether the trait is absent or present. If the trait is present, it is necessary to examine whether the trait's expression is constitutive or inducible (i.e., expressed in some environments but not others).

Beyond categorizing a plastic trait as constitutive or inducible, it is often desirable to quantify inducibility by scoring trait expression quantitatively across two or more environments, sometimes along a gradient. Quantification of trait expression in multiple environments can be useful for translating a trait's plasticity into a trait in and of itself, a trait that is necessarily quantitative. Quantifying plasticity as a continuous trait has been carried out by calculating measures of spread (e.g., variance, coefficient of variation) (Schlichting 1986) or by using the raw or standardized difference between contrasting environments (Falconer 1990; Ungerer *et al.* 2003). In some situations, the absolute value of differences is used. This may be an appropriate choice because it is clear that the direction or pattern of plasticity is bidirectional (i.e., passive phenotypic

plasticity, for which the range of responses is of greater interest than the directionality). Or it may be used because of insufficient knowledge about the details of a given syndrome of plasticity (Scheiner & Berrigan 1998; Dewitt & Scheiner 2004; van Kleunen & Fischer 2007). Researchers who already know that the magnitude of plasticity varies, and that it typically shifts in one direction (i.e., active phenotypic plasticity), tend to quantify a trait in two environments and to use the difference in trait values between environments as a metric of plasticity. In such systems, plasticity can also be examined across an environmental gradient, followed by fitting a reaction norm function and using the function's parameters (e.g., slope, intercept, higher-order curvature) to quantify plasticity (Finlay & Wilkinson 1963; Gibert *et al.* 1998; Stratton 1998; Stinchcombe *et al.* 2004; Kingsolver *et al.* 2007). Any quantification of plasticity allows for ranking genotypes in terms of greater or lesser "magnitude" or "level" of plasticity and for analyzing plasticity as itself a quantitative trait.

Both theorists and empiricists have had to make decisions about these and other methodological details, which sometimes assume substantial differences in the underlying biology or ecological function. This is an essential step required before developing or applying models addressing whether the evolution and maintenance of plasticity depends on the details of the environment. Intuitively, and consistent with many theoretical models, loss of plasticity and greater stability are generally favored in more stable environments, whereas plasticity is generally favored by heterogeneous or fluctuating environments—but outcomes can be complex depending on the reliability of the cue and/or the sensory detection mechanism that organisms use to detect environmental fluctuations, by time lags, by details of the plasticity-eliciting and selective environments, and by costs associated with plastic phenotypes (van Tienderen 1991; Moran 1992; Scheiner & Callahan 1999; Sultan & Spencer 2002; Zhang 2006; Kingsolver *et al.* 2007).

Indeed, as work in this vein has progressed, it has often been noted that heterogeneity in the environment is ubiquitous (e.g., Lechowicz & Bell 1991), yet plasticity is neither universal nor infinite. Indeed, many traits are stable or canalized rather than plastic, and the existence of substantial genetic diversity tells us that no genotype has evolved plastic traits so flexible that it can dominate in all environments (Tollrian & Harvell 1999; Pigliucci 2001). Theorists and empiricists have therefore often emphasized "benefits" and "costs" to account for why plasticity versus stability may be selected in different ecological contexts. Unfortunately, definitions and usage of the term *costs* are frustratingly idiosyncratic. Sifting through the many reports with "phenotypic plasticity" in the title or keyword list, one will find some arguing that "costs" contribute to natural selection favoring plasticity, others arguing the exact opposite, and some even arguing both. In this review, we aim to clarify some of this confusion while still following the lead of many theoretical and empirical researchers specializing in the evolution of phenotypic plasticity. We will examine two basic yet distinct types of costs. On the one hand are "costs of the phenotype" and on the other hand are distinct "costs of plasticity" that may accrue beyond costs of the phenotype.

Understanding and quantifying costs of the phenotype requires examining the evolutionary consequences of having one phenotype rather than another phenotype. That is, in a certain environmental context, a comparison between distinct phenotypes reveals different patterns of covariation between one or more quantified traits and some other distinct organismal function. Many good examples of phenotypic costs are found in the literature discussing antipredator defense traits in prey organisms. The potential benefit gained by expressing a defense trait may be offset by a cost—a decrease in an organismal function unrelated to avoiding, escaping, or resisting predators. A central question is whether plastic trait expression allows organisms to avoid "paying the price" of

an inappropriately expressed trait in an environment where that trait is not advantageous. Where this is the case, an evolutionary advantage can be gained by shifting from expressing defense traits constitutively to expressing them plastically only when there is risk of predation. Such costs are referred to by researchers investigating many other ecological interactions, using many other terms: *costs of defense*, *costs of resistance*, *costs of induction*, *ecological costs*, or even *direct costs* (Levins 1968; Lynch & Gabriel 1987; Tollrian & Harvell 1999; Agrawal 2001). Regardless of such details, in this review we categorize such costs as "costs of the phenotype" or "phenotypic costs."

Conceptually distinct from costs of the phenotype are costs of plasticity. One way to understand costs of plasticity is to consider genotypes that have the same phenotype within an environment yet differ in their plastic responses to a variable environmental factor and in fitness. In such a situation, there is no phenotypic variation, precluding phenotypic costs. Because this is rarely the case in nature, and because this cannot always be accomplished experimentally, plasticity costs are quantified using statistical tools. After taking into account covariation between a focal trait and a fitness-related trait, it is possible to examine residual variation in the fitness trait, variation for plasticity itself (rather than the trait), and covariation between the two (van Tienderen 1991; DeWitt *et al.* 1998; Scheiner & Berrigan 1998). Quantifying plasticity costs involves thinking about plastic traits in a complex manner: the trait itself (as expressed in one or more environments) and the plasticity of a trait (quantified using a variety of methods; see earlier discussion).

Although both plasticity costs and phenotypic costs are characterized one environment at a time, both can be examined in more than one environment across a set of environments. Within individual environments, a key consideration is the relationship between a trait's phenotypic cost and its plasticity cost because plasticity costs can offset phenotypic costs. Specifically, finding that a trait's plasticity is associated with a plasticity cost can potentially explain why the trait fails to be plastic (or shows suboptimal plasticity.) In this regard, plasticity costs can and should be examined in more than one environment, because simulation studies suggest that plasticity costs are not likely to counter the evolution of adaptive plasticity if they occur only "locally" but can be important if they occur "globally" (i.e., within only one environment across a set of environments rather than in all or most environments across a set of environments) (Sultan & Spencer 2002).

The theoretical work of Sultan and Spencer (2002) shows that it is possible to think about plasticity costs and their implications whether one is considering a trait's plasticity or a trait's stability. Commonly, one asks whether plasticity costs counterbalance across-environment selection favoring plasticity (i.e., by reducing phenotypic costs). Or if one is more interested in stability, one might flip the concept and examine whether the "cost of stability" or "cost of canalization" counterbalances across-environment selection favoring stability (Dorn *et al.* 2000; van Kleunen & Fischer 2007). In this review we will refer to this general phenomenon as a "plasticity cost," but the same concept has been described with different terms (e.g., "indirect cost"). It has also been parsed into specific subcategories such as genetic costs, maintenance costs, energetic costs, and sensing costs (van Tienderen 1991; Moran 1992; Newman 1992; DeWitt *et al.* 1998; Kussell & Leibler 2005).

Whether an investigator is studying phenotypic costs, plasticity costs, or both, the literature tends to focus on certain types of traits (generally, plastic traits) and on populations with certain types of genetic architecture (generally, genetic variation for traits and for the plasticity of traits, i.e., genotype–environment interactions). In general, when delving into this literature, one also needs to bear in mind that many studies of phenotypic costs have not addressed

plasticity costs. And some studies have focused on plasticity costs only, paying little attention to phenotypic costs.

As a result, we currently lack an integrative understanding of these two types of costs, but we have important evidence for guiding progress in this direction. With this objective in mind, we will highlight some recent work addressing phenotypic costs only, some addressing plasticity costs only, and some addressing both types of costs. We begin with work emphasizing phenotypic costs, most of it involving the study of traits in prey organisms—putatively adaptive predator-induced plasticity. A review of studies addressing this phenomenon is useful for demonstrating the many different factors that can complicate the quantification and interpretation of phenotypic costs, even when research into the issue of plasticity costs is postponed or ruled out as unimportant. Many different environmental factors can change simultaneously, multiple traits can show plasticity to this multifaceted variation, traits can show plasticity at different points in the life cycle, traits can vary in the time lags necessary for their plastic expression, and some traits can show reversible plasticity. By focusing narrowly on one type of plasticity research, we will illustrate why a sophisticated approach is essential for understanding just one type of cost: phenotypic costs.

After considering the many possible complications involved in detecting phenotypic costs, we turn attention to whether such complications can explain why plasticity costs have been detected in a few studies but found to be negligible in many others. We will highlight experimental strategies that have been particularly successful for detecting costs of plasticity. An interesting question is whether it is possible to link phenotypic costs and plasticity costs detected at the population level to the molecular mechanisms underlying traits and their plasticities. This question is of general interest even though pinpointing the gene or genes contributing to focal phenotypic traits (and their plasticity) is probably more tractable in model genomic organisms (e.g., *Drosophila melanogaster, Arabidopsis thaliana*).

Having introduced integrative approaches for quantifying phenotypic costs and plasticity costs, we will discuss some recent studies focusing on microbial systems. In well-studied microbes, most traits are plastic and many are amenable to environmental manipulations using laboratory culture techniques. Such traits include the use of particular sugars, the ability to synthesize amino acids *de novo*, the development of flagella for motility, the formation of dormant spores from vegetative cells, and the growth of fruiting body structures. All of these are examples of traits not constitutively expressed but induced in the appropriate environment(s). As well, for many microbes true "experimental evolution" studies can be performed for many generations, with large populations, and with replication. The potential to carry out such studies allows direct testing of theoretical models, particularly the prediction that variable versus static environments indeed select for or against plasticity, respectively. It is also possible to obtain highly detailed information about, for example, the genes, sensory proteins, enzymes, and biochemical pathways—in short, the molecular machinery responsible for phenotypic plasticity. As in work with other model organisms, work carried out with microbes allows direct insight into how a plastic trait and its environment-specific expression maps to specific genes, noncoding regulatory regions, or gene products within one or more biochemical pathways. Simultaneously, empirical data about population sizes or mutation rates make it possible to probe the effect of selection, mutation, and genetic drift on the maintenance or loss of plasticity and the associated genes regulating that plasticity. These examples should be of broad interest to researchers interested in reconciling the mechanistic details of plastic traits with the concepts of phenotypic costs and plasticity costs, concepts that originally emerged from more "black box" approaches to modeling the evolution of plastic traits.

Costly Antipredator Phenotypes: Past Success Stories, Future Challenges

For decades, prey organisms have been a favorite study system for investigators interested in understanding plastic traits from ecological and evolutionary perspectives. Prey organisms encounter variable and complex environments, exhibiting many different forms of plasticity in response to this variability. They often invest available time, carbon, energy, or other resources to different degrees into morphological and behavioral traits. Responses can be fairly general against certain types of predators (Van Buskirk 2001; Relyea 2004) or can be adaptive against one type of predator but maladaptive against others (Mikolajewski *et al.* 2006). In either case, certain patterns of resource allocation may be beneficial because they increase the ability to evade or resist predators. Behavioral responses—seeking shelter or reducing activity—reduce the probability of detection, encounter rates, or possibly both (Werner & Anholt 1993; Lima 1998). Morphological defenses—neck teeth, helmets, spines, bulgy bodies, shell shape, and thickness—increase the chance of prey survival in the event of an attack (DeWitt *et al.* 1999; Tollrian & Harvell 1999; Kishida & Nishimura 2004; Mikolajewski *et al.* 2006). Other morphological structures, such as larger tail fins, improve the ability to flee (McCollum & Van Buskirk 1996). Physiological defenses such as toxicity are well characterized in plant–herbivore systems but are little explored in animal systems, though some have been shown (Benard & Fordyce 2003). Finally, developmental responses in organisms with complex life cycles, such as earlier metamorphosis, may reduce the time in predator-vulnerable life stages (Tollrian & Harvell 1999). Indeed, there is an almost overwhelming diversity of induced defenses across taxa in many traits (Lima 1998; Tollrian & Harvell 1999; Lass & Spaak 2003; Relyea 2007).

For any given defense trait, the potential benefit gained by expressing the trait may be offset by a cost—a decrease in another important organismal function. Such functions might include efficient feeding, rapid growth rate, opportunities for mating, or defense against another type of predator. A common, general, verbal hypothesis is that natural selection favors antipredator traits that are inducible rather than constitutive because inducible traits accrue fewer costs in environments that vary spatially or temporally in predation risk. Formal optimality models are often developed to examine inducible defense traits, and often these theoretical models assume that defense traits are subject to time, energy, or other resource allocation tradeoffs (Abrams 1984; Werner & Anholt 1993; McNamara & Houston 1994; Lima 1998; Steiner & Pfeiffer 2007). When two (or a set of) induced responses are all continuous traits, the magnitudes of the plastic responses and various tradeoffs can be quantified, allowing estimation of the phenotypic cost of each response. This approach requires investigators to appropriately and explicitly define the trait or traits that are construed as costs—the "currency" of the cost (Steiner 2007a; Steiner & Pfeiffer 2007). Empirically, quantifying trait-specific costs, identifying how traits are integrated, and investigating which traits trade off against each other has been challenging (Van Buskirk 2000).

Research addressing phenotypic costs and tradeoffs has often progressed with nonlethal predator–prey experiments, involving caged predators in tanks, or mesocosms. Such studies provide insights that cannot be gained using free-ranging predators, in which the consequences of within-population variation in defense traits are often confounded with the consequences of competitive release (i.e., population density decreases and resource increases as predator-vulnerable individuals are removed from the population) (Van Buskirk & Yurewicz 1998; Relyea 2007). Similarly, in many plant–herbivore systems, it can be difficult to distinguish between an adaptive plastic response to herbivory and the negative effect of herbivory on growth, competitive ability, and fitness (but

see Agrawal *et al.* 2002). Freshwater predator–prey systems have been particularly useful because chemical cues often induce predator-defense traits, and these chemicals can be used to trick the prey organisms to express the full set of behavioral, life-historical, physiological, and morphological responses. It is possible not only to manipulate traits to assess and even quantify the extent to which the traits are plastic but also to remove the potential benefit of the plastic trait.

Such well-targeted experiments are essential for linking inducible traits to their potential costs, particularly when multiple tradeoffs act at the same time. In many organisms, the energetic cost of a response (i.e., the underlying energy or carbon allocated) cannot be measured directly, but instead each response is gauged relative to associated traits construed as costs. In organisms with complex life histories, accounting for costs can be particularly challenging because they accrue during a life cycle spanning multiple stages (Van Buskirk & Saxer 2001). Also, the time of initiation matters. For example, predator-induced responses occurring later during ontogeny may bear greater costs (Hoverman & Relyea 2007). Also, responses might be induced once and then maintained without much extra cost, or they might be cheap to initiate but costly to maintain (Tollrian & Harvell 1999). For instance, some predator-induced morphological defenses in *Daphnia* are initiated in the mother's generation, and once the morphological defense structure is built, it is cheap to maintain (Tollrian & Harvell 1999; Lass & Spaak 2003). In contrast, predator-induced morphological responses in tadpoles can be highly reversible, which is sometimes considered evidence that their maintenance is costly (Kishida & Nishimura 2004).

Many studies look for associations between continuous predator-induced phenotypes and for reductions in a known component of fitness or an assumed fitness proxy—reduced reproduction (Tollrian & Harvell 1999), reduced growth (Van Buskirk 2000), reduced rate of development (Tollrian & Harvell 1999; Relyea 2007), reduced immunity or reduced investment in fat storage (Stoks *et al.* 2006b), or increased mortality not caused by predation (Steiner 2007a). Simplistically, selection is expected to eliminate costs that markedly reduce an important fitness component. Yet such costs can persist because multiple costs or benefits are integrated at the whole-organism level (Stearns 1992).

It can be more challenging to explain why studies sometimes fail to detect cost of defense traits. Induced traits are expected to become fixed if they do not involve phenotypic costs (Via & Lande 1985), but many defense traits are induced by predators (or predator-related cues). However, costs might be eroded by selection over time, and studies may fail to detect them because they are infrequent or subtle. Also, empirical studies might overlook costs because they are not a major focus of the study. We use a review by Relyea (2007), who surveyed 41 studies constituting 29 amphibian species of predator-induced responses involving shifts in the time or size at metamorphosis in amphibian larvae, to illustrate these possibilities. Costs would be revealed by amphibians' metamorphosis being later and at a smaller size. There was no consistent evidence for costs. Whereas nine studies showed a phenotypic cost of responding to predators, three studies revealed unexpected positive fitness effects, and 10 studies showed combinations of costs and positive fitness effects, by being earlier and smaller (one study) or later and larger (nine studies). Despite comparable experimental setups and manipulative environments, half the studies revealed no evidence for phenotypic costs associated with either time to metamorphosis or size at metamorphosis.

These findings make it difficult to judge overall phenotypic costs. In amphibians, size at metamorphosis is mostly linearly correlated with subsequent survival, whereas the cost of delayed metamorphosis increases exponentially (Altwegg & Reyer 2003). Yet in half the studies that found later and larger metamorphs and measured early growth, there was

reduced growth early during ontogeny followed by increased growth later. Although such catch-up growth has fitness costs in fish (Metcalfe & Monaghan 2001) and damselflies (Stoks *et al.* 2006a), evidence for costs of catch-up growth or delayed costs in postmetamorphic stages of predator exposure during the larval phase has not (yet) been found in amphibians (Van Buskirk & Saxer 2001; Altwegg 2002; Nicieza *et al.* 2006).

Despite the ambiguity of costs between studies, results from studies using the same prey species and same predator species were consistent (Relyea 2007), indicating that variations in costs are unlikely to be experimental artifacts. Rather, there may be differences in costs between prey species and costs specific to the phenotypes induced by particular predators, which has been shown in other studies (Van Buskirk 2000, 2001; Relyea 2004). Variance in predator- and prey-specific costs might arise from differences in selection strength, which could lead to variance in erosion of costs. Also, different predators pose different threats to different prey organisms (Van Buskirk 2000, 2001; Relyea 2004). Also, between different prey and predator species, induced responses differ in their associated benefits or costs (Mikolajewski et al 2006). Some of the equivocal evidence for costs may be experimentally driven because costs are not necessarily linearly correlated across environmental factors (Steiner 2007a). Relyea (2007) reviewed experiments that were conducted with many different species and under a range of conditions, and there might have been considerable variation among the studies in how thoroughly the investigators quantified predator- and prey-specific relationships between environment-specific trait expression and the trait or traits construed as costs (e.g., growth, time, size at metamorphosis).

Evidence for costs during early ontogeny comes from one of the few studies that explicitly investigated cost of induced defenses by comparing 15 anuran species in their response to exposure to chemical cues from invertebrate odonate predators (Van Buskirk 2000). Growth costs were found in 13 species, and one of the species that showed no growth cost (*Hyla chrysoscelis*) showed high survival costs. This provides one possible answer to the question of why some studies focusing on only survival or only growth would fail to detect costs. To quantify and determine the origin of costs, Van Buskirk (2000) used an allocation tradeoff approach, with the expectation that the level of the (trait specific) defense should be reflected in the level of the cost. Contrary to the predictions, species that showed increased costs of defense did not show increased phenotypic responses in activity, body length, or tail depth, although there was a tendency for species that showed a strong reduction in activity when exposed to predator cues to show some additional decrease in survival. This lack of a correlation (between the magnitudes of responses and costs) suggests that comparisons among species might not be an appropriate way to detect clear relationships between induced traits and the magnitude of costs, perhaps because costs of the response differ between species, as suggested by Relyea's (2007) review.

Studies within one species that have related the magnitude of response to the magnitude of the phenotypic cost have also failed to reveal clear relationships. For instance, a study investigating induced defenses and their associated costs for *Rana temporaria* tadpoles along a resource gradient revealed overall costs of induced defenses in survival and development (Steiner 2007a). Along the resource gradient, however, there was no simple relationship between the induced defense and the traits construed as phenotypic costs of defense; these shifted along the gradient. At low resource availability, costs resulted predominantly in reduced survival, whereas at high resource availability, costs yielded a reduced development rate. A study where prey density (competition) was manipulated instead of resources led to the same conclusion: The level of the defense is not correlated with the level of the cost (Teplitsky *et al.* 2005). Despite clear evidence for costs of defense in these studies, defense traits and costs

were not linked in a simple, direct manner. This finding returns us to the challenge of whole-organism phenotypes consisting of many different traits.

The challenge of understanding costs and benefits of plasticity in an integrated framework may be partially solved by building models that investigate integrated trait responses with multiple defense traits and their associated phenotypic costs (i.e., Steiner & Pfeiffer 2007). Although such models are improvements compared with those examining one tradeoff, both rely on assumptions about tradeoffs that might not be met. For instance, a reduction in foraging activity, shown by reduced growth rates, is one of the most effective defense mechanisms (Werner & Anholt 1993; Lima 1998) but is also assumed to be one of the highest costs of defense. However, experiments that used different time lags to disentangle behavioral, physiological, and morphological responses show that tadpoles that reduce their feeding activity under (nonlethal) predator exposure did not reduce the amount of food ingested, evacuated the food from their guts at higher rates, and did not show reduced growth rates (Steiner 2007b). This finding shows that feeding activity is decoupled from ingestion and growth, potentially by physiological mechanisms such as differences in conversion rates or metabolic rates. Similar results were found in a comparison of two damselfly species (McPeek 2004). Greater activity in one species did not translate into higher feeding rates, and both species ingested the same amount of food, but high levels of activity led to higher predation rates. The species differed in the conversion rate of assimilated food under predation threats. In another experiment aimed at discovering underlying physiological mechanisms, it was confirmed that predator-induced shell morphology in intertidal snails is caused by an active increase in calcification rate (Brookes & Rochette 2007). Improved understanding of underlying physiological mechanisms is critical, because this knowledge can challenge common assumptions found in many models. It is therefore important to recognize the tentative nature of interpretations of single-species studies because they may involve implicit or explicit assumptions about the mechanisms of plasticity.

It may be time to move beyond simple correlation analyses between predator-inducible defense traits and their costs to exploration of the mechanism(s) underlying tradeoffs, possibly by taking advantage of established and emerging model organisms. The availability of good genetic information may permit exploring another potentially relevant issue: whether the same genes regulate both traits and plasticities, compared to separate genes regulating the plasticity of a trait but not the trait itself (e.g., Ungerer *et al.* 2003). This is an important issue because of its potential to influence whether traits and associated plasticities can evolve independently (Scheiner 2002; Callahan & Pigliucci 2005). Indeed, preliminary results in the *Arabidopsis* model system are promising, and in time it should be possible to deepen our understanding of the links between ecologically important traits (e.g., resisting or tolerating predators) to the underlying physiological mechanisms (Banta & Pigliucci 2005) or to quantitative trait loci (QTLs), the chromosomal intervals harboring quantitative trait genes (e.g., Weinig *et al.* 2003; also see review by Stinchcombe & Hoekstra 2008). Progress in attaining such longer-term goals may involve mapping of QTLs, followed by confirming that candidate genes harbored within QTL intervals are in fact the genes involved in regulating plastic traits. Comparable progress in the arena of antipredator traits may eventually be feasible, and the ease of crossing experiments in amphibians has already been demonstrated (Laugen *et al.* 2005).

Assessing Phenotypic Costs and Plasticity Costs with Genetic Model Organisms

Clearly, plasticity occurs not only in prey organisms subject to predation but also in many other species and in many other traits. Yet there

is no organism with a limitless ability to adjust its phenotype to match any and all environments (i.e., perfect plasticity). The hypothetical nature of such a phenotype, sometimes called a "Darwinian monster" (Pigliucci 2001), has often led investigators to broaden their focus to examine not only phenotypic costs but also plasticity costs as a type of cost separable from phenotypic costs. Both types of costs can prevent the evolution of perfect plasticity, and the two types of costs can reinforce or oppose each other.

A quantitative genetics framework is often used to estimate and test whether there is selection on plastic traits. In such analyses, one of the first and most basic goals is to test for genotype–environment interactions because this is evidence of genetic variation for plasticity and a prerequisite for its evolution (Via & Lande 1985; Via *et al.* 1995). Because genetic correlations within and among environments theoretically constrain the evolution of plasticity (Via & Lande 1985), scoring and analyzing multiple traits and multiple plasticities is a feature found in almost all published studies examining putatively adaptive phenotypic plasticity with these methods.

Investigators interested in plastic traits have modified classical methods for analyzing selection (Lande & Arnold 1983; Endler 1986), using them to determine whether the direction or strength of selection on a plastic trait differs among environments, an adaptive explanation for the evolution and maintenance of phenotypic plasticity (e.g., Via & Lande 1985; Dudley & Schmitt 1996). Two related methods are commonly used, and both involve examining correlations (as estimated by selection differentials or selection gradients) between relative fitness and traits. One type is an environment-specific analysis, which is performed "locally"—within multiple environments that evoke plasticity in at least one of the multiple traits. Another type is carried out across environments, or "globally"—estimates are obtained for each genotype's mean fitness by appropriately averaging across environments, and the same procedure is used to estimate each genotype's trait mean. It can be argued that across-environment analyses are unrealistic unless they can somehow incorporate information about the natural frequency of alternative environments, but both within-environment and across-environment analyses are often found in the plasticity literature. Both types of analysis address the issue of phenotypic costs (i.e., whether traits expressed via plasticity are positively correlated with fitness), often by comparing genotypes that vary widely in plasticity: Some may fail to strongly express a trait, some express the trait inducibly, and others express it constitutively.

Because neither analysis separates out plasticity costs per se, a third and complementary type of analysis is sometimes performed with plasticity cost analyses also conducted "locally" or within particular environments. This analysis examines whether there is selection for or against the plasticity of a trait (or traits) after accounting for selection acting on directly the trait(s) (van Tienderen 1991; DeWitt *et al.* 1998; Scheiner & Berrigan 1998).

Often, with data from one study, all three types of selection analysis can be carried out (DeWitt 1998; DeWitt *et al.* 1998), and it is frustrating that relatively few studies combine all three types. One example is Stinchcombe *et al.*'s (2004) reanalysis of data for the model plant species *Arabidopsis thaliana*. The data, originally collected by Westerman and Lawrence (1970), and previously reanalyzed by Lacey *et al.* (1983), focused on 21 inbred lines of natural ecotypes and for 12 mutant lines. Replicates of each genotypic line were grown in three different temperature environments.

After estimation of selection gradients within the temperature treatments, it was clear that the traits expressed by more plastic genotypes were associated with lower genotypic mean fitness than the traits expressed by less plastic genotypes. Also, more plastic genotypes had lower fitness averaged across environments than less plastic genotypes; that is, there was across-environment selection against plasticity. Both

results are evidence that phenotypic costs were not contributing to the maintenance of plasticity. Plasticity costs were found within two of the three temperature environments. From these three perspectives in combination, it seems that selection may favor reduction or elimination of temperature-evoked plasticity in this species—because of a combination of phenotypic costs that fail to maintain plasticity, combined with plasticity costs.

This issue brings us to a frequent and intuitively appealing argument: Selection ought to eliminate costly plasticity (DeWitt 1998; DeWitt *et al.* 1998). Such an argument has been upheld by simulation models (Sultan & Spencer 2002) and by many published studies reporting their failure to detect significant plasticity costs (Scheiner & Berrigan 1998). Counterarguments are many, however, because some studies have succeeded in detecting plasticity costs. First, as argued by Stinchcombe *et al.* (2004), selection may have a limited ability to eliminate maladaptive plasticity because it operates on multiple traits simultaneously. This may explain why their study found phenotypic costs associated with a plastic trait (i.e., maladaptive plasticity), as well as plasticity costs separated from phenotypic costs. Nonetheless, finding that a syndrome of plasticity is maladaptive may bias researchers against follow-up analyses to examine plasticity costs. While acknowledging this potential bias, van Kleunen and Fischer reviewed plasticity costs in plants (2005) and argued that costs have been detected often enough to merit continued investigation. These authors also discussed a second bias that may prevent studies from finding plasticity costs: the use of insufficiently challenging or realistic environments in experimental studies. Such treatments inflate environmental variance and therefore may obviate detection of selection either on plastic traits (i.e., by diminishing phenotypic benefits or costs) or on plasticity (i.e., by diminishing plasticity costs).

Although many authors have suggested that plasticity costs may be larger or easier to detect in harsher environments (e.g., Steinger *et al.* 2003), few studies have been designed to directly examine this idea. A notable exception is Steiner and Van Buskirk's work (2008) with tadpoles. In addition to rearing tadpoles in conditions that elicit an antipredator phenotype, they also independently examined if plasticity costs varied across treatments with either high or low intraspecific competition. Plasticity costs were not detected at the whole-organism level, and they were not consistently associated with either plasticity or stability when examined at the level of individual plastic traits.

One impediment to progress in understanding plasticity costs is the challenge of comparing studies. Each makes slightly different choices in quantifying plasticity and in deciding whether to focus only on active, adaptive plasticity and plasticity costs or to be more expansive and to consider phenomena such as passive plasticity, maladaptive plasticity, or costs of canalization (van Kleunen & Fischer 2007). Although reanalysis of past studies might be a productive project, doing so would require cooperation in archiving and compiling raw data from previous and ongoing studies. Another problem is that selection analyses are sometimes based on unrealistic or overly simplistic assumptions about the heterogeneity of plasticity-evoking environments and selective environments. Work by Stinchcombe *et al.* (2004), discussed earlier, involved across-environment selection analyses using simple, unweighted averages of genotypic trait means and genotypic means for fitness. Yet several theoretical and simulation studies have demonstrated that the response to selection depends on the details of both the plasticity-eliciting and selective environments, which may not be identical (Scheiner & Callahan 1999; Sultan & Spencer 2002; Zhang 2006). Unfortunately, few studies have attempted to characterize the spatiotemporal distribution of selective environments (Feder *et al.* 1997; Huber *et al.* 2004) or the environments known to trigger plastic responses (Sultan *et al.* 1998; Scheiner & Callahan 1999).

Even rarer are studies examining both phenotypic costs and plasticity costs in field experiments. However, field studies have detected plasticity costs associated with density-induced traits in annual mustards (Weinig *et al.* 2006; Dechaine *et al.* 2007). The findings of Weinig *et al.* are probably directly tied to the genetic material used rather than the organism, traits, or ecological contexts examined. As the authors carefully explain, their studies did not use genotypes drawn from natural populations. Instead, they used new experimental populations of segregating progenies—specifically, large populations of recombinant inbred lines. In natural populations, selection may have culled genotypes with unfavorable combinations of plasticity loci and fitness loci, an argument echoing those of DeWitt (1998) in his influential work on plasticity costs. In an experimental population of segregating progenies, some genotypes may have had fitness and plasticity loci in coupling phase (high plasticity, high fitness; low plasticity, low fitness), whereas others may have these loci in repulsion phase (high plasticity, low fitness; low plasticity, high fitness). Beyond this argument, the many distinct genotypes within such populations clearly increase statistical power for detecting plasticity costs, and transgressive segregation enhances genetic variance for traits, for plasticities, and for fitness (Callahan 2005; Weinig *et al.* 2006; Dechaine *et al.* 2007).

Studies with populations of recombinant inbred lines are potentially amenable to QTL analyses using traditional or array-based technologies. QTL mapping of chromosomal regions can be performed for pairs of traits, making it possible to examine whether there are individual loci (closely linked genes) that affect traits and fitness, traits and plasticities, plasticities and fitness, or some combination of these (Callahan *et al.* 2005). This is an intermediate step in pinpointing the specific QTL, as well as the possible function of these genes within regulatory pathways that detect environmental inputs and that regulate developmental or physiological responses to those inputs. With this information in hand, it will be possible to examine directly whether such genes contribute pleiotropically to fitness or perhaps interact with genes that contribute to fitness.

As previously argued by Agrawal (2001), knowledge of the genetic mechanisms of signal detection and response is necessary for properly interpreting plasticity costs (or their absence). He discussed three hypothetical genotypes: one plastic and two nonplastic. In one nonplastic genotype, the sensory and physiological machineries underlying plasticity are intact, except for a defect in a small, downstream step. Despite its static phenotype, it pays plasticity costs comparable to those incurred by the plastic genotype. The second nonplastic genotype derives its static phenotype from a nearly complete lack of plasticity machinery. Accordingly, it incurs much lower costs. Distinguishing among such alternatives requires integrative methods for characterizing plastic phenotypes, phenotypic costs, and plasticity costs not only at the population level but also at the level of genetic pathways operating within specific tissues and cells.

Interestingly, some of the earliest demonstrations of phenotypic costs and adaptive phenotypic plasticity, carried out in plants, involved comparing wild types possessing a plastic trait with mutant or transgenic knockout genotypes having a nonplastic phenotype—either lacking the trait or expressing the trait constitutively (Schmitt *et al.* 1995; Pigliucci & Schmitt 1999, 2004). Similar demonstrations were performed with *Drosophila melanogaster* genotypes genetically engineered to carry extra copies of genes for heat-shock protein 70 (HSP70) (Krebs *et al.* 1998). A particularly innovative yet often miscited and misinterpreted study (Krebs & Feder 1998) compared a *D. melanogaster* control line to a transgenic line altered to synthesize a "dummy" protein beyond the synthesis of HSP70. This strategy essentially tried to superimpose an additional metabolic and energy expenditure (i.e., protein synthesis), a cost beyond the normal HSP70 response. The researchers could then compare the control and altered

genotypes in currencies more relevant to evolution and ecological function: survivorship and time to metamorphosis. Their conclusion was that these energetic and metabolic costs were negligible.

Plant researchers have also focused on well-characterized heat stress response systems to conduct integrative experiments addressing phenotypic costs and plasticity costs (Larkindale *et al.* 2005; Tonsor *et al.*, 2008). Tonsor *et al.* focused on HSP101, which responds rapidly to temperature changes. Although there are multiple heat-shock proteins in plants, loss of HSP101 results in an inability to survive extreme heat stress. The study compared HSP101 loss-of-function null mutants and wild types under both thermally benign and stressful conditions. In stressful conditions, protein content varied among wild types carrying a functional gene, and this variation affected many phenotypic traits—above- and below-ground, vegetative and reproductive. Finding that the inducible HSP101 system can confer phenotypic benefits rather than phenotypic costs was especially intriguing because these phenotypic benefits differed between two different genetic backgrounds (the Columbia and Landsberg *erecta* lab strains), indicating strong epistasis. In benign conditions, loss of HSP101 functionality sharply reduced reproductive output, indicating pleiotropy.

In the same study, Tonsor *et al.* also surveyed 10 wild-collected accessions of *A. thaliana* drawn from a latitudinal gradient. They found significant variation in temperature response of HSP101 protein content, calling into question the notion that HSP101 is a consistently advantageous stress response system and raising the question of whether it is costly. Such a hypothesis has been previously advanced based on arguments about the energetic and metabolic demands required for synthesis of these proteins (e.g., Heckathorn *et al.* 1996). Having a costly HSP rapid response system may be advantageous, but over time more frequent exposure to heat stress and selection to minimize such costs may result in plants that cope with stress by using less costly alternatives. Yet a functional allele at the locus is maintained, perhaps because the gene has such pervasive pleiotropic effects.

Although it is informative to make comparisons among multiple contemporary populations, such studies entail implicit assumptions about the variable selection histories experienced by these populations. As already noted, the direct characterization of the plasticity-evoking potential and selective effect of heterogeneity in contemporary environments is challenging and has been attempted only rarely. Similarly characterizing the heterogeneity of past environments may be more problematic, if not impossible.

The limitations of such comparative and retrospective studies have led many researchers to use experimental evolution strategies, typically with rapid-cycling plants or animals (Scheiner 2002; Callahan 2005; Garland & Kelly 2006). Such studies can be limited by their short-term nature, small population sizes, or lack of replication. Accordingly, microbial organisms offer a particularly attractive system for using experimental evolution to pursue questions about the evolutionary processes contributing to phenotypic costs and plasticity costs.

Studying Costs with Microbes: Experimental Evolution and Genomic Approaches

It has been known for many decades that microbes cultivable in lab conditions are ideal for experimental evolution research strategies given their short generation times (20 min) and large population sizes ($>10^7$). This potential is also linked, for many different microbial species, to the availability of powerful genomic and transcriptomic databases and tools. Moreover, microbial phenotypes are astonishing in their diversity and versatility, often including traits important for nutrient uptake or metabolism, the ability to form spores in the face of nutrient scarcity, or motility and taxis

in response to a variety of stimuli. The phenotypes and genomes of microbes offer a powerful framework in which phenotypic traits, plasticity, phenotypic costs, plasticity costs, and their genetic basis can be addressed in a direct and integrative fashion. The remaining challenge is to understand the types of ecological contexts confronted by microbial organisms and to devise methods for mimicking and manipulating these contexts in the laboratory.

In nature, some environments may supply abundant macromolecules important for bacterial viability (e.g., nucleotides, amino acids), but in other environments these are naturally scarce. Such contrasting conditions can be mimicked in the laboratory by devising enriched media that include abundant macromolecules and contrasting them with media lacking one or more of these nutrients. Such laboratory methods can convincingly demonstrate that many bacteria can synthesize such macromolecules *de novo* from other, simpler sources of carbon and nitrogen and that these abilities are typically plastic rather than constitutive. When such macromolecules are present in the environment, expression of the proteins required for their *de novo* synthesis is typically repressed. When these macromolecules are not available, maintaining fitness requires induced expression of these proteins. Many such responses have been studied in microbes. In almost every example studied, the phenotypic response is tightly regulated, requiring detection and integration of external environmental cues and, in turn, adjustments in the expression of enzymes or other proteins involved in the relevant metabolic pathways (for details, see Neidhardt *et al.* 1996; Sonenshein *et al.* 2002).

Using a variety of microbial models, it is therefore feasible to undertake the new research strategy of determining whether plastic phenotypes are maintained or lost over evolutionary time (i.e., tens or thousands of generations) and to examine maintenance or loss of plasticity in a variety of environments. Such systems are ideal for examining whether there are plasticity costs distinct from phenotypic costs. This examination would not be carried out in an environment lacking an essential macromolecule, where a nonmutant parental genotype and a loss-of-function mutant genotype would differ phenotypically. Rather, such studies are performed in permissive, stable environments where both genotypes have identical metabolic phenotypes but might differ in fitness because of plasticity costs. If significant, such plasticity costs would result in mutant genotypes outcompeting and replacing their parent genotypes, taking over the population.

Such predictions about plasticity costs are in fact long-standing ones, and several previous microbial studies have tested them. Zamenhof and Eichorn (1967) isolated *Bacillus subtilis* mutants that could synthesize neither histidine nor tryptophan. Mutants were mixed and grown with their wild-type parent strains, in an environment containing the amino acid they could not synthesize, and these mutants dominated the population within ~50 generations. This finding suggests that mutations that result in loss of macromolecular synthesis are advantageous when that macromolecule is constantly available. However, the selective advantage associated with the mutant is puzzling because the presence of histidine or tryptophan should repress the expression of proteins required for their synthesis, so neither the mutant nor the wild-type strain is predicted to be expressing them. Furthermore, the mutants were not regulatory, so for leaky expression of unneeded proteins, the mutant would be expressing a nonfunctional protein. Therefore, it is unlikely that the advantage is due to a difference in production costs. A lack of plasticity costs associated with amino acid production was also supported by results from Dykhuizen (1978), who meticulously calculated the energy costs of making the amino acid tryptophan. He found that even though there were energy costs associated with making tryptophan, these were smaller than the selection differential observed between a mutant unable to make tryptophan and its wild-type parent.

Although both studies address whether there is a cost associated with environmentally regulated and induced macromolecular synthesis, we do not know if these lab-constructed mutants represent mutations that arise naturally. In a more realistic situation, where loss-of-function mutants arise spontaneously in a population, would selection favor an increase in their frequency? Would the loss of the ability to synthesize macromolecules trade off for the optimization of another phenotype? Maughan *et al.* (2006) addressed these questions in an evolution experiment where the bacterium *Bacillus subtilis* evolved in nutrient-rich medium for 6000 generations. Because this medium was rich in macromolecules that would otherwise need to be synthesized, it was not surprising that they observed a decline or loss in the ability of the population to synthesize all macromolecules that were required for growth in nutrient-poor medium.

Using experimentally measured populations parameters such as mutation rates, competitive fitness, and fitness components (e.g., growth rate), Maughan *et al.* (2006) looked for an association between the decline in macromolecular biosynthesis and increases in fitness to determine whether selection was responsible for the phenotypic loss. Although results from statistical analyses suggest a role for selection in phenotypic loss, the fitnesses measured experimentally did not correlate with phenotypic loss. Perhaps the fitness assays performed did not accurately measure fitness, or perhaps selection for phenotypic loss was too subtle to detect in the fitness.

Additional work using experimental populations of bacteria has also shown that selection favors the loss of inducible phenotypes, suggesting that the maintenance of these phenotypes is indeed costly. The decay of metabolic breadth over evolutionary time was documented in populations of the bacterium *Escherichia coli*, where it was found that the decay was attributable to tradeoffs, such that selection favored phenotypic decay (Cooper & Lenski 2000). Loss of the genes whose products catabolize ribose was also observed repeatedly in experimental populations of *E. coli*, suggesting that the loss of this phenotype provides a fitness benefit (Cooper *et al.* 2001).

The results from the experiments described suggest that selection favors the loss of inducible metabolic functions when these functions are not important for fitness. This assertion in turn suggests that these phenotypes are costly to maintain. Whether it is more costly to monitor nutrients in the environment or to regulate the expression of these phenotypes is not clear because usually the exact genes that are involved in phenotypic loss are not known. For the loss of the ability to catabolize ribose, this loss was due to the deletion of genes encoding the proteins for the transport and catabolism of ribose (Cooper *et al.* 2001). In most populations, the gene whose protein product senses ribose in the environment, and consequently induces expression of the proteins for ribose catabolism, was deleted. However, 2 of 11 deletions did not include the gene encoding the sensing protein, leaving it unclear whether the sensing function was costly and/or whether a different component of the phenotype was costly.

Most of the metabolic phenotypes discussed above are relatively simple, requiring only a few proteins for both environmental sensing and metabolism. The evidence shows that the maintenance of these phenotypes is costly when they are not important for fitness. An important question is whether loss of phenotypes of greater complexity would be more strongly advantageous. One of the most complex phenotypes in bacteria is the development of a vegetative cell into a spore, and this phenotype may have high physiological costs. Many pathways in the cell are committed to sensing environmental change, and spore development is initiated when the cell senses that nutrients are scarce. Environmental signals are integrated to increase the expression of early sporulation genes. Once sporulation has been initiated it cannot be stopped; spores can become vegetative again only if they complete the entire

sporulation process and then germinate. Bacterial spores are metabolically dormant and can withstand environmental assaults at a frequency much higher than that of vegetative cells.

Maughan *et al.* (2007) evolved 10 spore-forming populations of *B. subtilis* for 6000 generations, five with and five without selection for spore development. For those five populations without selection for sporulation, excess nutrients were added to the environment so that sporulation would not even be initiated, although presumably environmental sensing was still occurring. Thus, any selection would be predicted to remove plasticity costs associated with phenotype production, not sensing. After observing that sporulation ability declined or was lost in all experimental populations, it was necessary to determine whether mutation alone resulted in loss of this complex trait or if selection favored sporulation loss. To appropriately estimate corresponding selection coefficients, simulations were carried out to estimate rates of neutral mutation accumulation, another explanation for loss of sporulation. Simulations were conducted using an experimentally measured rate of mutation. There was clear evidence that selection favored the loss of sporulation ($s = 0.01$) in only one population. In the remaining four populations, simulations that incorporated selection were no better at explaining the decline in sporulation than those that assumed neutral mutation accumulation. This finding suggests that, even in a constant environment, sporulation was not a costly form of plasticity to maintain in most populations.

The lack of a cost associated with sporulation maintenance seems contradictory to the selective advantage that could potentially be associated with the complex function. Sporulation is a phenotype that relies on the ordered expression of hundreds of genes, and it might be expected to incur a larger cost than a metabolic pathway, a phenotype usually encoded by fewer than 10 genes. However, genes involved in the production of both sporulation and metabolism are expressed only when sensory machinery recognizes the appropriate environmental cue. Only genes whose products sense environmental cues are constitutively expressed. In contrast, expression of genes whose products are involved in trait development occurs after the cue has been sensed. Therefore, plasticity costs associated with environmental sensing are likely to be the same for all inducible traits, whether encoded by hundreds or tens of genes.

Work with the *B. subtilis* system has not yet explored the potential loss of sensory genes in benign and static environments. However, a parallel issue was addressed in a study (Kussell & Leibler 2005) that addressed two different phenomena often discussed as mechanisms for generating phenotypic variability within clonal populations of microbes: responsive switching or stochastic switching. These strategies map onto what plasticity researchers sometimes refer to as "active" and "bet-hedging" plasticity (Kaplan & Cooper 1984). Responsive switching is presumably more costly because it requires sensory mechanisms (i.e., a specific category of a plasticity cost) (see also Kussell *et al.* 2005). The work of Kussell and Leibler (2005) suggests that stochastic switching is likely to be favored over responsive switching when environments fluctuate only rarely.

A main strength of these microbial studies is their ability to focus narrowly on plasticity costs by experimentally eliminating phenotypic variation by using a benign and static environment and then using this context to investigate factors contributing to loss of plasticity-related genes such as sensing genes. As a result, studies have tended to emphasize plasticity costs per se, rather than phenotypic costs and plasticity costs occurring together. Yet phenotypic costs associated with inducible traits can be examined in microbes, as nicely illustrated in a recent study examining the loss of flagellum-based motility in *Pseudomonas fluorescens* (Hall & Colegrave 2008). Mirroring work with predator-induced traits in tadpoles, the study involved manipulating resource levels in the environment, combined with an experimental evolution approach. This strategy

was successful in demonstrating that evolutionary loss of motility occurred more rapidly in low-nutrient environments, where flagella were predicted to be more costly because of resource limitation, than in high-nutrient environments. This demonstration that flagellum development may be subject to costs that vary with resource availability is something that could be attempted with other traits such as sporulation ability or amino acid synthesis. A future challenge for work with microbes parallels the challenges facing those working with plant or animal systems: appropriately quantifying both cost of phenotypes and costs of plasticities, most likely by doing so over multiple environments.

Over the long term and in natural environments, there may be considerable ecological consequences associated with loss of traits such as sporulation or inducible metabolic pathways, because these traits are likely to be important determinants of niche breadth. That is, the complete loss of a plastic trait can result in a transition from being a generalist to being much more of a specialist. This was shown to be the case in experimental populations of *E. coli* that evolved for 10,000 generations on one carbon source (Cooper 2002). When each population was tested for its ability to use other carbon sources, growth on alternative carbon sources was on average lower. Furthermore, competitive fitness in four alternative environments was poor. These same *E. coli* populations also had reduced survival at different temperatures (Cooper *et al.* 2001). As in other organisms, however, interpretations of these results is contingent on how well the range of environments used in the laboratory reflect the actual range and frequency of environments encountered in nature (e.g., alternative carbon sources tested in the *E. coli* example or the nutrient availabilities used in studies of *P. fluorescens*).

All these examples have focused on a typical experimental evolution strategy in microbes: comparing genotypes in which an important functional trait is either inducible or lost altogether. Such a comparison is extremely useful for examining plasticity costs, allowing direct evaluation of how mutation and selection balance and contribute to either the maintenance or decay of a plastic trait. Sometimes comparisons can be made to examine costs of the phenotype. Studies carried out with traits that are either inducible or altogether absent would be well complemented by comparisons between genotypes unable to express a trait, able to express it only in certain environments, or expressing it constitutively. Although genetic manipulations in the lab could probably create microbial genotypes with constitutive expression, an important and interesting question is whether such genotypes could arise spontaneously. In either case, such genotypes could be useful for integrative microbial studies that expand beyond examination of plasticity costs in isolation to the study of plasticity costs combined with phenotypic costs.

Future Prospects

Whether working with animals, plants, or microbes, researchers investigating plastic traits will continue to require familiarity with the phenomena of phenotypic costs and plasticity costs and need to be guided by previous theoretical and empirical work addressing costs. We have tried to highlight the diversity of this vast and burgeoning literature, aiming to aid other researchers in discerning and developing creative and promising directions for future research.

For example, studies are increasingly asking questions about the mechanisms underlying plastic traits. This type of work will probably become increasingly important and feasible as more efficient and less expensive technologies are developed for examining multiple genes or proteins, including those involved in sensing and responding to environmental signals. Currently, such work tends to be confined to well-established model organisms, but this narrow focus is likely to broaden as the universe of genomic models rapidly expands. It is rapidly becoming feasible to apply these tools as well to ecological favorites such as black cottonwood

trees (Tuskan *et al.* 2006), the aquatic crustaceans *Daphnia* (McClintock & Derby 2006), and emerging models closely related to existing genomic models (Clauss & Koch 2006; Schranz *et al.* 2007).

Were Woltereck alive today, he could follow up his careful and ultimately frustrating observations of *Daphnia* reaction norms by zooming in, focusing on physiological or genetic mechanisms that account for the patterns of variation. For those interested in such an approach, there are clear steps to be taken. When studying *Daphnia* and its predators, it may be feasible to pursue these types of questions by using microarray-based studies of genetic variation or gene expression. At the same time, it is a globally widespread organism that can be investigated for basic ecological questions, as well as for more applied studies in phenotypic plasticity (e.g., ecotoxicology). Finally, although scarcely comparable to bacteria, *Daphnia* can nonetheless be grown in lab culture conditions in large populations with short generation times.

Another emphasis in our review, and something highlighted by other commentators, is that it may be critical to understand not just the molecular genetics but also the population genetics underlying plasticity costs. This notion, emerging from studies of experimental populations (e.g., recombinant inbred lines), is something that can also be explored by working with experimentally manipulated hybrid genotypes or with naturally occurring hybrids, such as hybrid species and their progenitors or genotypes in hybrid zones. Such approaches will allow exploring the importance of phenotypic plasticity in maintaining species boundaries or making these boundaries permeable to gene flow. They will also be generally useful for investigating how phenotypic costs and plasticity costs contribute to the processes that affect the evolution of specialists and generalists (Lexer & Fay 2005; Picotte *et al.* 2007; Pinkhaus *et al.* 2007).

The genomics revolution notwithstanding, ecologists are continuing to study plasticity by using fairly traditional methods. In the coming decade we expect to see many studies of plasticity motivated by the importance of understanding invasive species (Lee 2002; Richards *et al.* 2006) and how natural populations will respond to climate change (Bradshaw & Holzapfel 2006). Such studies may serve as opportunities to refine our understanding of how plastic traits function and evolve, including the roles of phenotypic costs and plasticity costs.

There will also be some work examining plasticity in a wider diversity of organisms and in a phylogenetic framework. We are talking not so much about comparative studies focusing on a few species (Schlichting & Levin 1984; Pigliucci *et al.* 1999) but on much more ambitious surveys that are phylogenetically informed (e.g., Nicotra *et al.* 2008). Phylogenetically informed analyses may also be required when attempting to synthesize existing studies of plastic traits (e.g., Kembel & Cahill 2005), something that has not yet been done in published meta-analyses of phenotypic costs and plasticity costs (Relyea 2007). Nonetheless, two main messages are clear from early meta-analyses: There is evidence for phenotypic costs, even though they are not universal, but evidence for plasticity costs is equivocal. In part, this may reflect the fewer studies examining plasticity costs or a bias against publishing negative evidence. It may also stem from the difficulty of sifting through reports that have made arbitrary or inconsistent (or unstated) decisions about how to define, quantify, and analyze phenotypic costs and plasticity costs. It may also reflect a failure to recognize the difficulty of interpreting plasticity costs without information about the phenotypic costs associated with a trait (i.e., whether selection favors loss of the trait, plasticity of the trait, or constitutive expression) (also see van Kleunen & Fischer 2007). Finally, more studies of plasticity in microbial organisms are forthcoming and should not be overlooked. This work is stimulating because it reminds us that plastic traits are affected not just by selection but also by mutation, drift, or migration. It will be interesting to see if those working with plants or

animals can devise and execute research that directly examines how these different processes, together with phenotypic costs and plasticity costs, jointly contribute to the evolution of plastic traits.

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Conflicts of Interest

The authors declare no conflicts of interest.

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