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INVITED REVIEW

Fundamental links between genes and elements: evolutionary implications of ecological stoichiometry

PUNIDAN D. JEYASINGH and LAWRENCE J. WEIDER
Program in Ecology and Evolutionary Biology, Biological Station and Department of Zoology, University of Oklahoma, Norman, OK 73019, USA

Abstract

Organisms require elements to live and reproduce. We already know that availability of certain elements [e.g. phosphorus (P)] is highly variable spatiotemporally. In addition, there is variability in demand for various elements ontogenetically, as well as phylogenetically. Nonetheless, we know little about the underlying causes for such variation. In this study, we surveyed the literature to identify genes involved in the homeostasis of one biogenic element, P. Evidence from the literature suggests that variation in the environmental supply of P affects expression of highly conserved genes (e.g. the phosphate transporter system). In addition, we found evidence for genetic variation in the acquisition, assimilation, and allocation of P. Such effects of P supply should impinge on fitness, and drive evolutionary change. Further understanding of the inter- and intraspecific fitness consequences to imbalances in the availability of P in relation to other biogenic elements may be useful to disentangle primary mechanisms driving diversifications. We argue that studying the interactions between stoichiometric constraints and underlying genes is relevant to the majority of organisms, and conclude by furnishing information on designing and interpreting experiments using this approach.

Keywords: elemental composition, integrative biology, microevolution, natural selection, phosphate transporters, phosphorus-responsive genes, phosphorus

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Ecological stoichiometry: an integrative framework to study microevolution

While biologists have focused on the causes and consequences of striking differences in morphology, anatomy, and genetics among organisms, at the atomic level, organisms also differ in their elemental composition. All organisms are composed of multiple elements held together by various chemical bonds that are formed and broken during primary biotic processes (e.g. anabolism, catabolism; Williams 1997). During development, offspring rely on their genetic machinery to harvest, process, and store different elements [e.g. carbon (C); phosphorus (P)] from the environment to live and reproduce, in a variety of different ways (e.g. autotrophs, heterotrophs), and hence differ, for reasons not yet fully established, in their somatic elemental composition. Often, the elemental composition of organisms is quite different from that of their habitat (Williams & Fraùsto da Silva 1995; Sterner & Elser 2002). Mismatches in elemental composition between diet and somatic requirements elicit an array of responses across a broad range of organisms from bacteria to vertebrates and at many key levels of organization (e.g. regulation of homologous genes, growth, reproduction), and as such should aid in integrating eco-evolutionary mechanisms in a phylogenetic context. Ecological stoichiometry (Sterner & Elser 2002) is an integrative organizational framework that has helped us appreciate the importance of simultaneously considering multiple elements (or such elements encompassing biomolecules), with predictions at several hierarchical levels of organization (Elser *et al*. 2000b; Sterner & Elser 2002). While this approach has catalyzed considerable work at higher levels (e.g. ecosystem level nutrient cycling), its evolutionary relevance

Correspondence: Punidan D. Jeyasingh, Department of Ecology, Evolution and Behavior, University of Minnesota, 1987 Upper Buford Circle, St Paul, MN 55108, USA. Fax: (612) 624 6777; E-mail: jeyas001@umn.edu

at the individual (i.e. fitness) level is still underappreciated (see Kay *et al*. 2005; Elser 2006). An elemental approach should enable us to identify common selective factors that affect key processes at multiple levels that impinge on fitness of most genomes and organisms. While we are rediscovering the role of the environment in evolution (e.g. West-Eberhard 2003), such general definitions of the environment will promote compatibility of data and hypotheses both horizontally (across taxa), and vertically (across levels of organization) — a central challenge in 21st century science (i.e. consilience *sensu* Wilson 1998).

Despite the striking similarity in the elements involved in basic life processes (i.e. the elemental composition of substrates and enzymes involved in metabolic reactions such as glycolysis) among all organisms, species and genotypes within species vary in their somatic elemental composition (e.g. DeMott *et al*. 2004; Frost *et al*. 2006). Furthermore, there is evidence of differential performance of species and genotypes under contrasting elemental supply environments (see below). Sources of such variation can be behavioural, physiological or developmental in nature — all of which are orchestrated by a combination of genetic and environmental drivers (e.g. Carroll *et al*. 2001). Characterization of these genetic and environmental drivers underlying variation in somatic elemental content and subsequent observations on their fitness relevance could serve as an ideal vehicle to integrate microevolutionary processes operating at different levels of organization (Fig. 1). However, such endeavours first require information on the genetic variation in acquisition, assimilation, and allocation of various biogenic elements. Although almost all elements in the periodic table have some biological role, the mass of living systems is largely comprised of six elements called the major biogenic elements, namely, C, H, N, O, P and S. In addition, based on their abundance in living systems, there are a few other elements classified as minor, and trace biogenic elements. These elements underlie organic reactions that are implicated in the origin of life. Here we surveyed the literature to see whether there is intraspecific genetic variation in the ability to sequester, process, and distribute P in a variety of organisms. Although studies explicitly analyzing genetic variation and its fitness consequences in response to P availability were scarce in our survey of the literature, there is reason to believe that such genetic variation may be widespread (e.g. Beebe *et al*. 2005). Further studies on the origin and maintenance of such genetic variation in the handling of these key elements may provide a mechanistic basis for explaining diversifications in the genomes of diverse taxa over geological timescales. For example, recent work (Elser *et al*. 2006) indicates marked effects of elemental supply environments (focusing on the relative supply of nitrogen, compared to other biogenic elements) in disparate genomes that have generated biases in the usage of various amino acids (differing in the number of N atoms in their side chains) in proteomes of plants and animals. A similar effect of trace metal availability through geological time has been observed in the metallomes of prokaryotes and eukaryotes (Dupont *et al*. 2006). Finally, there is evidence indicating that organisms may be capable of adapting rapidly to elemental supply environments. Specifically, Bragg & Wagner (2007) found preferential expression of low-carbon proteins under carbon-limiting conditions in yeast, which has the potential to dictate genome and transcriptome evolution. Clearly, genome properties and environmental elemental supply are coupled. Such fundamental links could be made relevant to the majority of organisms given an operational framework.

Our goal in this review is to introduce and demonstrate the utility of elemental approaches in evolutionary ecology by: (i) highlighting the evolutionary importance of one key element, phosphorus (P); (ii) discussing the broad classes of genes and gene complexes involved in the handling of P in diverse organisms; (iii) focusing specifically on the response of one family of genes (P transporters) under contrasting P supply environments; (iv) briefly discussing some responses occurring above the genetic level; (v) summarizing the literature on the intraspecific genetic variation in responses to altered P environments; and (vi) concluding with a short primer on designing and interpreting such studies in any organism. Consequently, this conceptual overview is by no means exhaustive, and we apologize in advance for any omissions of studies, without which much of the cited work would not have been possible. It should also be noted that genetic variation in other key responses at the individual level, such as behavioural (e.g. Bertram *et al*. 2006), morphological (e.g. Gahoonia & Nielsen 2004), and life-history (e.g. Jeyasingh & Weider 2005) adaptations in response to P supply should also play an important role in determining evolutionary potential of various species and genotypes. Such adaptations and responses to P availability are not covered here for brevity. Interested readers are directed to Sterner & Elser (2002), Kay *et al*. (2005), and many key references therein for a detailed treatment of these important topics, although genetic information on these topics is scarce. Here, we focus on whether there is genetic variation in performance under various P-defined environments, and identify putative loci that may be underlying such fitness differences.

The microevolutionary relevance of P

We have chosen to focus our review on the supply of P, because, although scarce in the known universe and biosphere (micromolar concentration; Macia 2005), P is ubiquitous, and performs critical functions within living systems (often in millimolar concentrations). This observation has led chemical evolutionists to implicate cometary

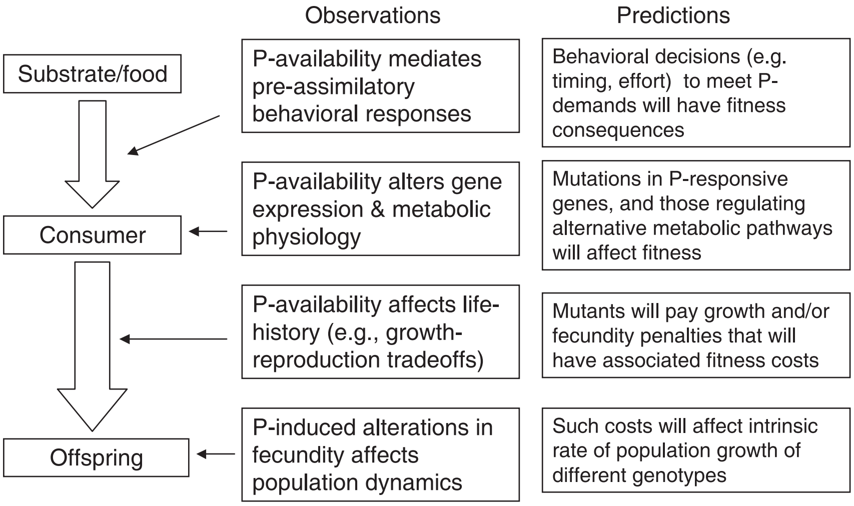

Fig. 1 Conceptual schematic showing the observed responses of organisms to environmental P supply at various levels of organization, with corresponding predictions on their fitness consequences.

delivery of P as a precursor for the origin of early biomolecules, because comets and meteors have higher concentrations of P, among other physical and chemical attributes relevant for primordial biosyntheses (Llorca 2005). The physico-chemical properties of P predispose it to be a critical component of many key compounds (Westheimer 1987) that carry out basic biochemical processes such as: nucleic acids that transmit genetic information, nucleotides that are precursors in DNA and RNA synthesis, phospholipids that make up the cell membrane, sugar phosphates which are key molecules in carbohydrate metabolism, and adenosine tri-phosphate (ATP) as a primary cellular energy source. Furthermore, P also plays important structural roles such as in vertebrate skeletons, scales, etc. These central roles of P in the structure and functioning of primary biological components suggest that the lack of P, and the subsequent structural and functional consequences in these basic cellular components could affect individual fitness. Such a critical role of P in biological processes is surprising because of its aforementioned scarcity compared to other elements in the biosphere (Macia 2005). Nonetheless, for the same reason, it is not surprising that P deficiency is pervasive. Organisms have evolved a variety of responses to P deficiency, which can be observed at multiple levels of organization. Furthermore, strong evidence for genetic variation in the sequestration, and handling of P are beginning to appear in diverse organisms (e.g. common bean, Beebe *et al*. 2005; water flea, Weider *et al*. 2004). Specifically, Beebe *et al*. (2005) found 26 different quantitative trait loci (QTL) for P accumulation, most of which were correlated with root architectural traits which enhance the ability to sequester P from the soil. Identifying the functional relevance of such loci underlying fitness differences will enable us to integrate proximate mechanisms and explore how higher-order processes such as ecosystem-level nutrient cycling affects evolutionary trajectories of various organisms, and genotypes. For example, Weider *et al*. (2004) found that the structure of the ribosomal DNA (rDNA) intergenic spacer (IGS) and growth performance were correlated in three species of *Daphnia*. They discussed these results invoking the functional significance of rDNA introns in the P-intensive process of ribosome biogenesis (i.e. the *growth rate hypothesis*, Elser *et al*. 1996). Such links have since been found to be pervasive (Weider *et al*. 2005a). Clearly, there appears to be intrinsic links between P supply and genes. Such links may be of immense interest to evolutionary ecologists especially in the light of anthropogenic alterations to the sinks, and fluxes of P (Smil 2000).

Genes that respond to changes in environmental or extracellular P availability

The first noticeable genetic response to differences in environmental P availability is via P-responsive genes (a group of genes encompassing the *PHO* regulon, e.g. Wanner 1993). Genes that respond to P deficiency are generally classified as 'early' or 'late' responding genes. 'Early genes' are those that respond rapidly and non-specifically to P deficiency (e.g. ribo-regulators, transcription factors, and general stress responsive genes; Hammond *et al*. 2004; Sugiura & Ferraris 2004; Misson *et al*. 2005). 'Late genes' are those that alter morphology, physiology or metabolism under prolonged P stress (Hammond *et al*. 2004). Our understanding of the *PHO* regulons of bacteria (e.g. Wanner 1993) and autotrophs (e.g. Wykoff *et al*. 1999; Hammond *et al*. 2003) indicate that the *cis*-regulatory elements in the promoters of *PHO* regulon genes are important in genetic responses to P deficiency. Similar *cis*-regulatory elements have been identified in the *PHO* regulons of: yeast (Oshima *et al*. 1996), tomato (Liu *et al*. 1997), the legume *Medicago truncatula* (Burleigh & Harrison 1999), *Arabidopsis* (Martin *et al*. 2000), and rice (Wasaki *et al*. 2003). These *cis*-regulatory elements control expression of orthologous genes that encode short, nonconserved open reading frames rapidly, and specifically to P deficiency, and as a result act as ribo-regulators that control the function of molecules such as RNA, DNA or proteins (Martin *et al*. 2000; Wasaki *et al*. 2003). Therefore, one likely pathway through which organisms realize and respond to P limitation is via the genes encompassing the *PHO* regulon (see Werner & Kinne 2001). This pathway can be validated using recent genomic studies that document the global genomic response of model organisms to P limitation. All of these studies report up- or down-regulation of hundreds of genes in diverse organisms (e.g. *Escherichia coli,* Wanner 1993; *Chlamydomonas* & Grossman 2000; *Arabidopsis,* Misson *et al*. 2005; rice, Shinano *et al*. 2005; rainbow trout, Sugiura & Ferraris 2004) in response to the P environment. In addition, a recent review by Tenenhouse (2005) shows the importance of phosphate (Pi) transporters in the maintenance of P homeostasis in mammals (i.e. mice and humans). Clearly, P availability plays an important role in the regulation of a variety of genes (Table 1), in particular P transporter genes (Table 2), which affect expression of several critical phenotypes, and as a result may have significant fitness consequences (Table 3). In the following section, we highlight the role of P transporter genes in various organisms, and discuss their fitness relevance.

P transporter genes and their eco-evolutionary relevance

As discussed above, P limitation elicits marked (large-scale) genetic responses in a variety of organisms (Table 1). Moreover, several recent studies (e.g. Wykoff *et al*. 1999; Wu *et al*. 2003; Chang *et al*. 2005) have identified new P-responsive genes in diverse organisms. While summarizing all of these genes, and their effects on various organisms is beyond the scope of this paper, the general functions of these genes and their responses to P limitation (Table 1) may be useful to highlight their eco-evolutionary importance. In this section, we focus on the literature surrounding the role that Pi-transporter genes play in P acquisition and P homeostasis in various organisms (Table 2). In bacteria and plants, the transcription levels of high-affinity phosphate transporters enhance the ability to acquire and transfer phosphate under P limitation (e.g. Wanner 1993; Raghothama & Karthikeyan 2005). In animals, the Na-Pi cotransporter (NaPi-II) expression in the kidney, intestine and pyloric ceca appear to play a major role in the regulation of P homeostasis (Sugiura & Ferraris 2004; Tenenhouse 2005). Of critical importance is that homologues of these high affinity Pi transporter genes have been identified in diverse organisms ranging from archaea to higher vertebrates including humans (Saliba *et al*. 2006), and all these proteins have remarkably conserved functions (Werner *et al*. 1998; Werner & Kinne 2001).

Mutations in these transporter genes are known to have major consequences in P acquisition, transport, and processing in both plants and animals. For example, Shin *et al*. (2004) showed that P acquisition was drastically decreased in *Arabidopsis* carrying mutations in two major Pi transporters (specifically, the *pht1; 1* and *pht1; 4*). Similarly, Tenenhouse (2005) reviewed the literature surrounding mutations in NaPi-II cotransporters (i.e. Npt2a) and their effects on renal P wasting and regulation in mice and humans. In general, mutants failed to show adaptive increases in the expression of NaPi-II cotransporters in the renal brush border membrane (BBM) in response to P limitation. Such mutations are also thought to play important roles in clinical conditions of hereditary hypophosphatemic rickets with hypercalciuria (HHRH), and age-related bone loss. Such studies highlight the potential fitness impacts of phosphate transporter genes in various organisms. In summary, expression patterns of P transporters are strongly related to extracellular Pi concentration in all organisms studied hitherto (Werner & Kinne 2001). More specifically, Raghothama & Karthikeyan (2005) reviewed the plant literature and found highly synchronized up-regulation of P transporters under P-deficient conditions. In addition they report preferential up-regulation of P transporters in the roots, the primary anatomical organ in the sequestration of inorganic P from the rhizosphere. Similarly, Sugiura & Ferraris (2004) report experimental work on rainbow trout again indicating up-regulation of P transporters under P limitation. Furthermore, they report preferential up-regulation in the pyloric ceca, and kidneys, primary anatomical structures in the sequestration, and retention of P in vertebrates. Understanding the variation in expression patterns (e.g. timing, magnitude) of these genes in various genomes, and genotypes within a genome, in response to P limitation offers an ideal framework for studying both the ecological relevance and evolutionary significance of a fundamental environment-gene(s) interaction that is common to all organisms.

Effects of P availability at the cellular level

In addition to altered regulation of several genes such as P transporters, environmental P availability also invokes several alternative metabolic pathways. Alterations to metabolic pathways in autotrophs under P limitation have been well-studied. Generally, autotrophs alter their metabolic physiology to increase acquisition, retention, and processing efficiency of P (e.g. Tillberg & Rowley 1989; Schachtman *et al*. 1998; Raghothama & Karthikeyan 2005). These changes in metabolic pathways are achieved by increasing activity and/or transcription of proteins involved in alternative respiratory pathways (Harder & Dijkhuizen 1983; Plaxton & Carswell 1999; Hammond *et al*. 2004; Raghothama 2005). Furthermore, plants under P-limitation

Table 1 Some examples of relevant P-responsive genes in diverse organisms. NA, not available

Organism	Genes or proteins characterized	Primary reported functions	Reported response to P limitation	References
Saccharomyces cerevisiae (brewer's yeast)	PHO12	Secreted acid phosphatase	Up-regulated > threefold	Wu *et al*. 2004
	ZIP2	Meiotic recombination and disjunction	Up-regulated > threefold	
	VTC1	Suppressor of CDC42	Up-regulated > threefold	
	PHO5	Acid phosphatase precursor	Up-regulated > threefold	
Arabidopsis thaliana (thale cress)	Undescribed genes	Cell wall biosynthesis genes; possibly involved in lateral root expansion	Up-regulated	Misson *et al*. (2005)
	Photosystem (PS)-I and PS-II, Calvin cycle; Chlorophyll A/B binding proteins	Photosynthesis and nitrogen assimilation	Down-regulated	Wu *et al*. (2003)
	Ribosomal protein genes	P-starvation response; protein synthesis and degradation	Down-regulated protein synthesis; up-regulated protein degradation	
	SQD1	Sulfolipid synthesis	Up-regulated	Hammond *et al*. (2003)
Trichodesmium sp. (cyanobacterium)	PhoA, PstS1, PstS2	Involved in P-regulated scavenging mechanisms	NA	Orchard *et al*. 2003
Lycopersicon esculentum L. (tomato)	TPSI1	P-starvation response	Up-regulated	Liu *et al*. (1997)
Medicago truncatula (legume)	Mt-4	P-starvation response	Up-regulated	Burleigh & Harrison 1997
Oncorhynchus mykiss (rainbow trout)	CYP24	Cytochrome hydroxylase in vitamin D metabolism	Up-regulated	Sugiura & Ferraris (2004)
	VDR	Vitamin D receptor	Up-regulated	
	PiUS	Phosphate kinase	Up-regulated	
Sus scrofa domesticus (domestic pig)	CALCR	Calcitonin receptor	Up-regulated	Hittmeier *et al*. 2006
	VDR	Vitamin D receptor	Up-regulated	
	IGFBP3	Insulin-like growth factor	Up-regulated	
	IL6	Interleukin-6	Marginally up-regulated	
	TFIIB	Transcription initiation factor	Marginally up-regulated	
	SOX9	Sex determining region	Marginally up-regulated	
	OXTR	Oxytocin receptor	Marginally up-regulated	
	IGF1	Insulin-like growth factor	Up-regulated	
Various	Various	Inorganic phosphate (Pi) transporters	Up-regulated	See Table 2

Table 2 Some examples of genes involved in transport of inorganic phosphate (Pi) in various organisms. NA, not available

Organism	Genes or proteins characterized	Primary reported functions	Reported response to P limitation	References
Escherichia coli (enteric bacteria)	Pst A/B/C; Pit	High (Pi)-affinity membrane transporter	Up-regulated	Elvin *et al*. (1986); Wanner (1993)
Plasmodium falciparum (malarial parasite)	PfPiT	Na-dependent Pi transporter	NA	Saliba *et al*. (2006)
Crocosphaera watsonii (cyanobacterium)	PstS and several others	High-affinity phosphate binder; and several other functions	PstS was not expressed under P-sufficient conditions	Dyhrman & Haley 2006
Saccharomyces cerevisiae (brewer's yeast)	PHO84	High-affinity Pi/H + transporter	Up-regulated > threefold	Wu *et al*. 2004
Chlamydomonas reinhardtii (green alga)	Psr1	High-affinity Pi transport system	Up-regulated 10-fold	Wykoff *et al*. (1999)
Tetraselmis chui (marine phytoplankton)	TcPHO	High-affinity Pi transporter	Up-regulated ~10-fold	Chung *et al*. 2003
Spirodela oligorrhiza (duckweed)	Undescribed	High-affinity Pi transporter	Up-regulated	Hase *et al*. 2004
Arabidopsis thaliana (thale cress)	Pht1; 4	Pi transporters	Up-regulated	Misson *et al*. (2005)
A. thaliana	AtPT1, AtPT2	Pi transporters	Up-regulated	Muchhal *et al*. (1996)
Oryza sativa (rice)	OsPT11	Pi transporter associated activated during arbuscular mycorrhizal symbiosis	No response	Paszkowski *et al*. 2002
Caenorhabditis elegans (nematode)	Six membrane permeases	Pi transporters	NA	Werner & Kinne (2001)
Drosophila melanogaster (fruit fly)	Na-dependent phosphate cotransporter	Pi transporter	NA	MacIver *et al*. 2000
Oncorhynchus mykiss (rainbow trout)	NaPi	Na-dependent Pi transporter	Up-regulated 10-fold	Sugiura & Ferraris (2004)
Mus musculus (house mouse)	NaPiIIb	Na-dependent Pi transporter	NA	Hilfiker *et al*. (1998)
Homo sapiens (human)	NaPi-III	Na-dependent Pi transporter	NA	Busch *et al*. (1995)

bypass phosphate-dependent reactions in glycolysis such as ADP-dependent pyruvate kinase (Theodorou & Plaxton 1993), using PEP carboxylase and PEP phosphatase, which are also thought to contribute to P recycling within the cell (Theodorou *et al*. 1991). It should be noted that the induction of these alternative metabolic pathways is species-specific (Gonzàlez-Meler *et al*. 2001), indicating that some species may not be able to cope with prolonged P limitation (i.e. they pay a fitness cost for lacking such alternative metabolic pathways). We know much less about the metabolic responses of animals to P limitation. P deficiency in animals often arises when energy (carbon, C) is in surplus, while P is limiting protein synthesis. Such situations are common in nature, and animals have evolved metabolic adaptations to solve the problem of excess C (compared to P; Anderson *et al*. 2005). Recent studies indicate that consumers feeding on such imbalanced diets (compared to their soma requirements) preferentially excrete the molecule or element in surplus (i.e. carbohydrates, Zanotto *et al*. 1997; carbon, Darchambeau *et al*. 2003). Furthermore, there is evidence that consumers uncouple ATP synthesis from biochemical or mechanical work under such imbalanced diets (i.e. diet-induced thermogenesis; Westerterp 2004). The uncoupling of ATP synthesis from oxygen consumption in the mitochondria under imbalanced diets is carried out by specific uncoupling proteins in mice and humans (Ricquier & Bouillaud 2000). Mutations in the sequences coding for these uncoupling proteins are thought to be involved in obesity (e.g. obesity factors; Lowell & Spiegelman 2000). Furthermore, a recent study (Warbrick-Smith *et al*. 2006) demonstrated that animals (i.e. caterpillars of the diamondback moth, *Plutella xylostella* L) are capable of adapting to imbalances in the environmental supply of certain molecules (in this case, C-rich carbohydrates), by changing metabolic pathways after only a few (eight) generations of artificial selection. This resulted in shifts in oviposition preference based on the macronutrient (C) content of the host plant, thus leading to population divergence. These observations suggest that organisms have the potential to rapidly respond to variation in the relative supply of elements or molecules, which can lead to microevolutionary changes. Such intricate linkages among key elements is highlighted in a recent review by Hermans *et al*. (2006), where they show significant roles of C-rich molecules such as carbohydrates (sucrose) in the signaling of P limitation, and in the alteration of C allocation to roots which enhances P sequestration from the environment. The standing genetic variation for such adaptive capacities in natural populations remains to be ascertained.

Genetic variation in the handling of phosphorus

While we know that certain genotypes or species perform better under high P conditions (e.g. Grover 1989; Jeyasingh & Weider 2005), we know little about the standing genetic variation in natural populations for growth performance under contrasting P regimes. A few studies have demonstrated considerable variation in relative performance of genotypes (i.e. fitness over a few generations of experimentation), and strong genotype × P–environment interactions at the intraspecific level (see Table 3). For example, Weider *et al*. (1997) found that human-induced changes in P loading into lakes (i.e. eutrophication) over the past 40 years dramatically altered the genetic composition of the zooplankter *Daphnia galeata*. This study clearly indicates that P availability and its concomitant effects on food quality (e.g. cyanobacteria; Hairston *et al*. 1999, 2001) can be a strong selective force affecting the genetic structure and microevolutionary trajectories of populations. In another study, Jeyasingh & Weider (2005) found significant variation in life-history reaction norms of two genotypes (clones) of *Daphnia pulex* under contrasting P-supply environments, which subsequently altered susceptibility to predation. These results indicate that the amount of P available can strongly impact the direction and magnitude of genotypic selection. Furthermore, variation in the demand for P in populations of *D. pulex* from Wisconsin and Alaska could be driven by geographical and climatic factors (Elser *et al*. 2000a). Because of the short growing season, daphniids from Alaska are faster growers, and had higher somatic percentage P to support such rapid growth. It is reasonable to hypothesize that such diversifications occur due to considerable standing genetic variation in phosphorus acquisition and/or use efficiency (PUE) in ancestral populations. In another example, Beebe *et al*. (1997) showed that PUE of various common bean (*Phaseolus vulgaris*) genotypes had a geographical component. Specifically, they found significant differences in PUE among different ancestral lines. Furthermore, wild genotypes had much lower PUE indicating (artificial) selection for PUE after domestication. Targets for such artificial selection and source for variability in genotypic performance may be a few critical P-responsive genes (Table 1). However, such associations remain largely untested. It is reasonable to hypothesize that similar P-dependent effects on the performance and maintenance of genotypes may be occurring in other species as well.

Using ecological stoichiometry to probe microevolutionary mechanisms (Fig. 2)

We know much about how specific model organisms respond to P availability, at multiple levels of organization (e.g. gene expression, metabolism, morphology; Tables 1–3). Furthermore, there is evidence indicating genetic variation for such responses (Table 3). In order to make this information gathered on a few model organisms widely compatible, we need a mechanistically grounded framework capable

Table 3 Some examples of genetic variation in fitness correlates of a diversity of organisms in response to P-availability

Organism	Genotypes tested	Reported response to P environment	References
Heterotrophic bacteria (undescribed)	Two strains isolated from a single lake	Differential growth kinetics in laboratory cultures related to level of P in medium under different pulsed-regimes	Vadstein (1998)
Arabidopsis thaliana (thale cress)	25 inbred homozygous lines	Strong genotypic differentiation in initial P-uptake rates, the ability to deplete phosphate to low concentrations, specific root length, and root: shoot allocation variables.	Krannitz *et al*. (1991)
A. thaliana	Two accessions and their hybrid	F_1 hybrid showed enhanced P acquisition via heterosis of key morphological (root and root hair length) and physiological (phosphate transporter expression) characters	Narang & Altmann 2001
A. thaliana	Five accessions	Significant variation among accessions in root morphology, phosphate uptake kinetics, organic acid release, rhizosphere acidification, and the ability of roots to penetrate substrates.	Narang *et al*. 2000
Hordeum vulgare (barley)	24 cultivars	Significant opposing responses among cultivars in shoot dry matter, P concentration, acid phosphatase activity, root length and P influx into roots	Römer & Schenk (1998)
Commelina erecta (perennial herb)	Five clones	Genotype × P–environment interactions in total dry mass, leaf area, specific leaf area, leaf area ratio, P concentration in leaves and roots, P-absorption rate, and photosynthetic rate	Urich *et al*. 2003
Phaseolus vulagaris (common bean)	16 genotypes	Considerable variation in the degree of root angle response among genotypes	Bonser *et al*. (1996)
	Four genotypes	Variation in allocation to roots, and root respiration	Nielsen *et al*. 2001
	Two parental genotypes	Variation in root architecture and P acquisition	Beebe *et al*. (2005)
	Four genotypes	Differences in stimulation of adventitious roots	Miller *et al*. 2003
	Two parental genotypes	Variation in root mass, and root P content	Araujo *et al*. 2005
Cajanus cajan (pigeon pea)	21 genotypes	Variation in P sequestration and P-use efficiency	Vesterager *et al*. 2006
Lotus glaber (birdsfoot trefoil)	Two populations (ecotypes)	Variation in growth response to P fertilization	Kade *et al*. 2003
Panicum virgatum (Alamo switchgrass)	30 genotypes	Variation in P uptake, somatic P concentration, and growth	Missaoui *et al*. 2005
Saccharum spp. (sugarcane)	24 genotypes	Significant genotype × P–environment interactions in stalk weight, stalk number, and sugar yield	Glaz *et al*. 2000
Zea nays (maize)	Two parental genotypes	Variation in root hair length (including plasticity), and seed P content	Zhu *et al*. 2005
Triticum aestivum (bread wheat)	Four genotypes	Variation in P uptake, root and shoot mass in high P environments only	Valizadeh *et al*. 2002
	39 genotypes	~30% variation in relative shoot growth among genotypes, ~20% variation in P efficiency, genotypes showing higher P efficiency had higher absolute shoot dry weight under P deficient conditions.	Ozturk *et al*. 2005
Triticum durum (durum wheat)	34 genotypes	~30% variation in relative shoot growth among genotypes, ~30% variation in P efficiency, genotypes showing higher P efficiency had higher absolute shoot dry weight under P deficient conditions.	Ozturk *et al*. 2005
Daphnia pulex (water flea)	Two genotypes differing at the *Pgi* locus	Heterozygotes outperformed (growth, reproduction), and out-competed homozygotes in high-P conditions, and vice-versa.	Jeyasingh & Weider (2005); Weider *et al*. 2005b
D. galeata	Three clones	Approximately 0.05 to > 0.15 variation in the intrinsic rate of increase (r day^{-1}) among clones between low and high P conditions	Seidendorf *et al*. (2007)
D. galeata × *cucullata* hybrid	Three clones	Intrinsic rate of increase (r day^{-1}) among clones between low and high P conditions varied approximately between −0.02 and 0.05	Seidendorf *et al*. (2007)
Sus scrofa domesticus (domestic pig)	Two sire lines	P-deficient diet decreased weight gain of only one of the sire lines	Hittmeier *et al*. 2006

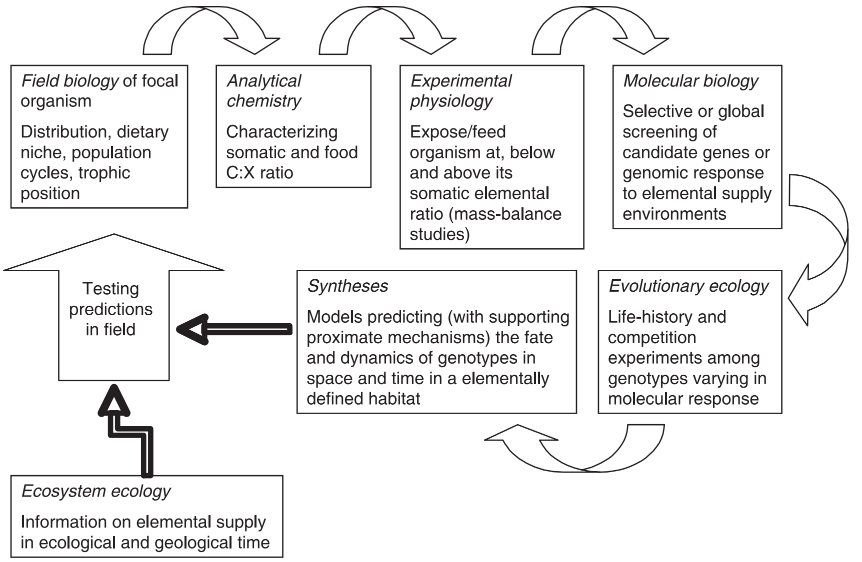

Fig. 2 Conceptual schematic demonstrating the different phases of a study using the framework of ecological stoichiometry.

of generating hypotheses with specific predictions via inductive reasoning. Ecological stoichiometry may be one such framework because it concerns the balance of energy and elements between the environment and individual. Therefore, by understanding the nature of the relationship between demand and supply for a biogenic element in a particular genome, we can exploit the growing genomic information of several model organisms to postulate hypotheses for any species. For example, does variation in inorganic P transport (via Pi-transporters) work in concert with the capacity to synthesize ribosomes (via the rDNA; Weider *et al*. 2005a) to produce distinct growth phenotypes? Such inductive approaches should complement deductive approaches such as QTL mapping to isolate the genetic basis of observed phenotypic differences.

In order to understand the nature of the relationship between demand and supply of a given element for a given species, we need basic information on the metabolic physiology of organisms in question. The threshold elemental ratio (TER; Sterner & Hessen 1994; Sterner & Elser 2002) is the elemental ratio consisting of at least two key elements, where limitation of metabolism, somatic and/or population growth changes from one element to another. TER for a particular element of various organisms can be calculated using basic information on an organism's metabolic physiology and the somatic elemental content of some other element, as shown by Frost *et al*. (2006) for determining the C:P TER of various aquatic organisms. Once relevant TERs are obtained, environmental supply of these elements can be readily manipulated in the laboratory to present these organisms with contrasting elemental environments (i.e. situations below, at, and above TER) enabling us to observe responses at different levels of organization (Fig. 1). Note that Frost *et al*. (2006) found striking patterns in the relationships between TERs, trophic levels, maximum growth rate and taxonomy. These results indicate that TERs may be a result of adaptive evolution. Whether signals of such adaptive mechanisms to the relative supply of biogenic elements can be observed on a phylogenetic tree, remains to be tested (but see Quigg *et al*. 2003; Hendrixson *et al*. 2006). Using the framework proposed here, we could generate and test hypotheses about the relationship between TERs of organisms, and genes that may be responsible for variable TERs. For example, is there a pattern in the genetic structure of P transporters among P-rich organisms with a low C:P TER, and low P organisms with high C:P TER?

Such information on the response of distantly related genomes will enable us to generate and test hypotheses that may help us begin to explain evolutionary diversification and specialization, not only at the microevolutionary scale using information from ecosystem ecology, but also at macroevolutionary scales using geological information. For example, Werner & Kinne (2001) show that the emergence of an important membrane transport system, NaPi-IIa is correlated with the origin of the mammalian nephron. It could be possible that such systems and structures were selected for because they enhance P sequestration and retention. Whether such evolutionary phenomenon can be mapped onto P availability in geological time remains to be seen. As such, this line of thought should generate integrative hypotheses (e.g. ecosystem impacts on gene expression and vice versa) that will advance our quest to understand the origin, functioning and diversification of life, especially in the 'omics' era where elemental information at the cellular level is becoming readily available. Similar integrative studies using other biogenic elements such as nitrogen, sulphur, etc. in the coming years should further our quest for a holistic understanding of how ecology shapes evolution.

Conclusion

With the capability to globally screen gene regulation in an increasing number of model organisms (i.e. functional genomics; Ferea *et al*. 1999; Townsend *et al*. 2003; Misson *et al*. 2005), we can readily identify genes and gene complexes that potentially impinge on fitness under various conditions (field, and/or laboratory). Analysis of such studies using a general conceptual framework discussed in this study should enable us to utilize genomic information from model organisms for a more thorough study of other organisms. For example, our work and the work of colleagues on various species and genotypes of *Daphnia* indicate variation in performance under elementally defined environments (Jeyasingh & Weider 2005; Weider *et al*. 2004, 2005b; Jeyasingh 2007; Seidendorf *et al*. 2007). Identifying the genetic basis for such phenomena is traditionally done using an open-ended QTL approach. The framework proposed here will enable us to make mechanistic predictions on putative loci (e.g. P transporters) underlying such differences using information gathered on organisms as diverse as bacteria and humans. Understanding evolutionary mechanisms at the elemental level, as proposed here, is an approach that is complementary to previous approaches that are often centred on the most apparent selective forces (e.g. ecological selection on morphology; sexual selection on colour pattern) within a particular system. While the conceptual framework described here does not encapsulate all the idiosyncrasies that are salient features of biological systems, it is a feature of such integrative models that have much to gain by understanding the causes and consequences of such idiosyncrasies (Elser & Hamilton 2007). Nonetheless, the core of such an elemental framework (Fig. 1) will still be consistent with first laws, regardless of the study system. An elemental approach to biology (e.g. ecological stoichiometry; Sterner & Elser 2002) transcends vertical (i.e. cross-discipline) and horizontal (i.e. cross-taxa) affinities, and is a powerful tool to improve compatibility of data and hypotheses in ecology and evolutionary biology as we proceed into the 21st century.

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P.D. Jeyasingh is a postdoctoral associate in the Department of Ecology, Evolution and Behavior (EEB) at the University of Minnesota. His doctoral research examined how the supply of phosphorus in relation to energy (carbon) impinges on traditional energy-explicit eco-evolutionary paradigms ranging from metabolic allometry to life-history evolution and trophic interactions. L.J. Weider is a Professor of Zoology and Director of the University of Oklahoma Biological Station. His recent collaborations have helped elucidate some of the underlying genetic (e.g. candidate genes such as rDNA) links between elemental ratios (primarily C:N:P) and impacts on the population genetic structure and microevolutionary potential of organisms.
