

## Toward a stoichiometric framework for evolutionary biology

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Kay, A. D., Ashton, I. W., Gorokhova, E., Kerkhoff, A. J., Liess, A. and Litchman, E. 2005. Toward a stoichiometric framework for evolutionary biology. – *Oikos* 109: 6–17.

Ecological stoichiometry, the study of the balance of energy and materials in living systems, may serve as a useful synthetic framework for evolutionary biology. Here, we review recent work that illustrates the power of a stoichiometric approach to evolution across multiple scales, and then point to important open questions that may chart the way forward in this new field. At the molecular level, stoichiometry links hereditary changes in the molecular composition of organisms to key phenotypic functions. At the level of evolutionary ecology, a simultaneous focus on the energetic and material underpinnings of evolutionary tradeoffs and transactions highlights the relationship between the cost of resource acquisition and the functional consequences of biochemical composition. At the macroevolutionary level, a stoichiometric perspective can better operationalize models of adaptive radiation and escalation, and elucidate links between evolutionary innovation and the development of global biogeochemical cycles. Because ecological stoichiometry focuses on the interaction of energetic and multiple material currencies, it should provide new opportunities for coupling evolutionary dynamics across scales from genomes to the biosphere.

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One of the principal insights of Darwin's theory of evolution by natural selection is the common descent of all organisms. Perhaps the strongest evidence for common descent is the shared underlying biochemistry based on nucleic acids, proteins, lipids, and other biomolecules. At the same time, natural selection has generated a plethora of morphologies, life history strategies and other differences that reflect variation in the way that organisms take up and transform energy and a variety of material elements. Ecological stoichiometry provides a framework for linking this variation to species interactions, food web dynamics, and nutrient cycling (Sturner and Elser 2002). In this paper, we expand the scope of ecological stoichiometry by exploring how evolutionary

processes have shaped and are affected by the elemental composition of organisms and their resources, and describe potential links between these processes and the patterns of adaptive variation in the biota.

Organismal stoichiometry (OS), which refers to elemental ratios in organisms, can be related in two ways to phenotypic and genetic variation upon which natural selection can act. First, OS reflects the biochemical characteristics of traits that affect functional performance and ecological interactions. Thus, anatomical or biochemical innovations that provide fitness benefits are manifested in stoichiometric changes. Second, stoichiometry reflects an organism's demand for resources, and failing to meet these demands can negatively impact

Accepted 24 October 2004

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ISSN 0030-1299

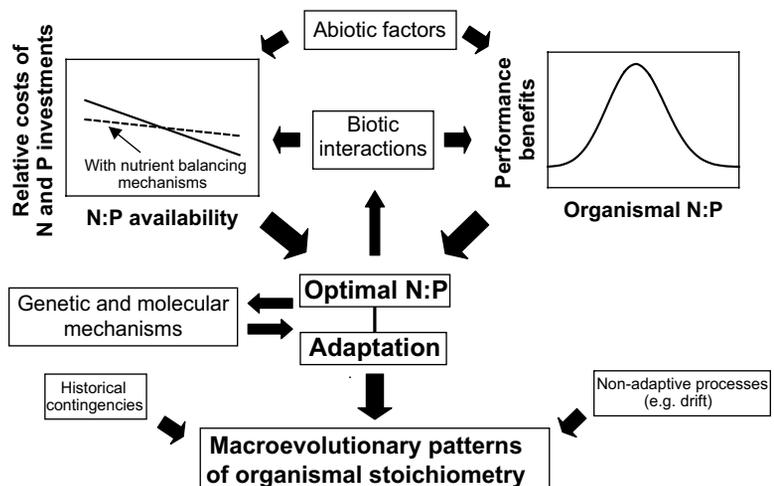
fitness. Thus, OS entails the fundamental evolutionary tradeoff between the material and energetic costs of a particular trait or strategy and its fitness benefit. Put another way, the stoichiometry of an organism represents the outcome of differential investments of various materials in structures that provide biochemical or biomechanical returns in the form of reproductively valuable work (Fig. 1). Because ecological stoichiometry focuses explicitly on multiple energetic and material currencies, elucidating these evolutionary tradeoffs in a stoichiometric context should provide promising opportunities for bridging evolutionary, ecological and biogeochemical studies.

To understand how selective regimes shape stoichiometry, the relationship between elemental composition, phenotypes, and functional performance must first be established. One such relationship, and a current centerpiece of ecological stoichiometry, is the growth-rate hypothesis (GRH), which predicts that selection on growth rate will tend to increase whole-body P concentration due to the role that allocation to P-rich ribosomal RNA plays in meeting the protein synthesis demands of rapid growth (Elser et al. 2003). The GRH likely has widespread evolutionary implications because all organisms share ribosomes as the principal engines of

growth, direct and indirect selection on growth rate is common, and P is often limiting in the environment. However, the same logic could be used to generate a wide variety of evolutionary hypotheses based on: 1) the stoichiometric signature of a trait important to a particular target of selection, and 2) the limited availability of important constituents determining that signature. As long as allocation of the limiting substance to the selected trait makes up a significant fraction of the organism's overall quota, increased uptake and conservation of this substance should occur. In this sense, ecological stoichiometry could provide a framework to better operationalize hypotheses of 'evolutionary escalation' (Vermeij 1994) which emphasizes the importance of investment in "mechanical structures" (sensu Reiners 1986) such as skeletons, woody tissues, shells, spines, and antlers, used to secure and defend resources, space and mates. These structures often differ substantially in elemental composition from the rest of the organism and thus represent a significant stoichiometric signal.

Ecological stoichiometry emphasizes measurable energetic and material currencies that can link environment, phenotypes, and diversification. Although the influence of resource availability on adaptive strategies has a long history in the study of evolution (Endler

Fig. 1. Diagram summarizing major relationships between organismal stoichiometry and evolution described in the text. We use nitrogen:phosphorus (N:P) for illustration, although other elements, energy storage molecules, etc. could be incorporated into the scheme. Here, the optimal N:P ratio in an organism (i.e. that maximizes reproductive success) depends on the relative costs of N and P accumulation and subsequent investment (left graph), and the functional consequences of trait composition and elemental requirements (right graph). Cost of investment depends on the relative environmental availabilities of N and P and mechanisms organisms possess for maintaining nutrient balance (e.g. selectively ingesting and retaining rare nutrients). Various abiotic factors and biotic interactions can affect nutrient availability and thus investment costs; they also can create tradeoffs and constraints that link functional performance to organismal N:P ratio. Adaptations linked to N:P stoichiometry may in turn affect the form of biotic interactions. Adaptive evolution of N:P depends on shifts in genetic and molecular mechanisms, which can themselves constrain N:P evolution. Finally, selection on organismal stoichiometry combines with historical contingencies and non-adaptive processes to generate macroevolutionary patterns.



1977), the task of determining how different factors shape the relationship between stoichiometry and fitness, and especially how these relationships feed back onto the abiotic environment, is more difficult, and few synthetic studies exist. In this paper, we begin with a consideration of the molecular and genetic determinants of OS. Then, we discuss the environmental factors that can influence the evolution of stoichiometric phenotypes. In the context of these internal and external factors, we then consider biotic interactions and how a stoichiometric perspective can generate new mechanistic hypotheses for explaining their adaptive significance. Finally, we explore the ramifications of a stoichiometric perspective for patterns of macroevolution and their long term reciprocal interactions with global biogeochemical cycles. In each section, we begin with a brief review of relevant, mostly recent, work that highlights the potential synthetic power of a stoichiometric approach to evolution. This approach is new and we highlight several exciting open questions in each section that may help chart the way forward. Throughout, we emphasize that adaptations generally represent compromises among multiple selective factors and our aim is to explore stoichiometric hypotheses as complementary evolutionary explanations, rather than as strict alternatives.

## Molecular and genetic determinants of organismal stoichiometry

To understand the evolutionary dynamics of growth rate, nutrient requirements and other traits with strong stoichiometric underpinnings, one needs to know more about the relationships between the genetic constituents and cellular machinery of growth and nutrient processing, and how they evolve under varying nutrient conditions. The bulk elemental composition of different classes of bioorganic molecules differs substantially. In particular, amino acids and proteins have high nitrogen (N) content but contain little or no P, while nucleic acids (RNA and DNA), coenzymes (e.g. thiamine pyrophosphate), and adenosine triphosphate (ATP) that serves as the primary energy currency of the cell are N-rich and especially P-rich and thus have low ratios of N:P compared to most other biomolecules (Sternler and Elser 2002). As a result, selection favoring different mixtures of biomolecules within organisms could produce substantial evolutionary changes in OS.

Recent work has begun to reveal some of the molecular and genetic mechanisms underlying stoichiometric evolution. The association of P-rich rRNA and growth rate has drawn attention to the role of rRNA genes (the rDNA) in supporting elevated production of rRNA for rapid growth (Cluster et al. 1987, Elser et al. 2000a). For example, variations in rDNA genotype, i.e. rDNA copy number (White and McLaren 2000) and the

structure of the intergenic spacer (Cluster et al. 1987, Gorokhova et al. 2002) have been implicated as responsible for differences in rRNA synthetic capacity and concomitant variation in P content and growth rate. In eukaryotes, the increase in repetitive DNA, and in particular rDNA, contributes largely to genome size variation (Prokopowich et al. 2003); amplification and unequal crossing over have been invoked to explain fluctuation of rDNA copy number. Because duplicate genes both increase the diversity of gene expression in an organism and evolve faster than do single-copy genes (Gu et al. 2004), the increase in repetitive rDNA may play an important role in the evolution of growth rate and ecological tolerance related to nutrient requirements. Indeed, genome size variation is correlated with environmental conditions and geographic distributions in a variety of species (Bottini et al. 2000) and can be manipulated under laboratory conditions (Nardon et al. 2003). However, high rDNA copy number does not necessarily mean that all these copies are functionally active – some of them could be selectively silenced by mechanisms that involve chromatin modification (Pikaard 2000), potentially complicating association between rDNA copy number, rRNA phenotype, growth capacity, and OS.

On the functional genomic level, differences in gene expression are central to the various mechanisms that regulate transcription and thus mediate the production of rRNA and proteins (Barker and Gourse 2001). There are several lines of evidence that nutrient deprivation elicits dramatic changes in gene expression patterns (Zinke et al. 2002, Stover 2003, Wu et al. 2004). For example, using *Saccharomyces cerevisiae* transcriptome data, Wu et al. (2004) showed that under nutrient imbalance, i.e. carbon (C), N, P, and sulfur (S) limitations and constant growth conditions, the majority of down-regulated genes fell into three functional categories: cellular organization, transcription, and protein synthesis. Interestingly, most down-regulated transcription and protein synthesis genes are involved in the synthesis and processing of rRNAs and ribosomal proteins, and thus may have a significant link to body N and P concentrations. Although this variation in regulatory mechanisms may be physiologically important, to be evolutionarily relevant, it must (1) be present in natural populations, (2) be heritable, and (3) lead to differential lifetime reproductive success. Regarding the role of this nutrition-sensitive regulatory variation in adaptive evolution, Townsend et al. (2003) found considerable variation in gene expression levels in natural populations of yeast, Brem et al. (2002) demonstrated the heritability of transcription, and Ferea et al. (1999) showed rapid change in gene expression in response to selection. These results argue for close attention to the role that regulatory variation may play during adaptive stoichiometric evolution. Elucidating the mechanisms

that underlie nutrient-modulated gene expression and its evolutionary implications will require the integration of knowledge and research methodologies from both nutrition and genetics.

At the level of biochemical constituents, shortages of particular elements may select for reductions of those elements in assimilatory peptides (Baudouin-Cornu et al. 2001, 2004). For example, selection during periods of nutrient limitation may explain why, in both *Escherichia coli* and *S. cerevisiae*, enzymes involved in S and C assimilation contain fewer S and C atoms per residue side chain than do average proteins in these organisms. Moreover, changes in elemental composition of proteins may be related to shifts in DNA composition and thus influence the evolution of the genetic code (Bragg and Hyder 2004). For example, variation in protein carbon content is tightly correlated with guanine and cytosine base composition in the genome, perhaps because DNA composition is a direct consequence of the optimization of protein atomic content in response to carbon availability in the environment (Akashi 2003, Baudouin-Cornu et al. 2004).

These emerging studies indicate that the time has come to undertake a comprehensive synthesis linking ecological genomics with ecological stoichiometry in an evolutionary framework. There is solid evidence for the existence of substantial and rapidly generated genetic and molecular variation related to elemental composition within and between species and populations, yet fundamental questions remain. It is essential to understand how changes in genomes and proteomes are governed by selection on growth-related traits, how such changes are translated into phenotypic functions, and what determines the extent of stoichiometrically relevant variation in genomes and macromolecules across taxa. New tools, including transcriptome, proteome and metabolome concepts, could allow a comprehensive assessment of whether and how genes and their functions evolve under stoichiometric constraints.

## Environmental factors

At the organismal level, biomass stoichiometry may reflect traits that influence functional performance in response to abiotic and biotic challenges. For example, abiotic factors such as season length, temperature, ultraviolet (UV) radiation, and ozone levels may be a significant part of the selective regime that shapes the elemental composition of organisms. Growing season length and other time constraints could influence OS via selection for faster development. For example, Elser et al. (2000b) showed that the microcrustacean *Daphnia pulicaria* from Alaska has a faster growth rate and higher P content than *Daphnia pulex* from a temperate region and suggested that the short arctic growing

season has selected for rapid development. Temperature has been invoked to explain increases in leaf nutrient concentrations in terrestrial plants that occur with altitude (Körner 1989) and latitude (Reich and Oleksyn 2004), although it is not yet clear the extent to which these patterns reflect intrinsic differences among species, adaptive phenotypic plasticity, or non-adaptive differences. Acclimation to low temperature can also result in increases in N, P, protein, and RNA concentrations in poikilothermic animals: such increases may reflect adaptive mechanisms for offsetting temperature-induced reductions in reaction rates or for enhancing cold hardiness, or they may simply reflect changes in the relative rates of macromolecular synthesis and degradation (Woods et al. 2003). In addition, elevated levels of UV radiation (Xenopoulos et al. 2002) and ozone (Pausch et al. 1996) can lead to stoichiometric changes in plants that mediate the stress of exposure. However, work is needed to determine whether these changes in ecological time reflect evolutionary patterns.

While the link between OS, abiotic factors, and fitness remains relatively speculative, the relationship between resource abundance and the evolution of OS has been more thoroughly explored. After all, it seems intuitive that lower demand for a particular nutrient may be selected for when that nutrient is scarce (Baudouin-Cornu et al. 2001, Fagan et al. 2002, Jaenike and Markow 2003). For example, leaf nutrient concentrations are generally lower in terrestrial plants that dominate nutrient-poor sites than they are in species from fertile areas (Aerts and Chapin 2000). Differential responses to increasing CO<sub>2</sub> and nutrient supply in plants may also have strong impacts on fitness, because CO<sub>2</sub> supply can alter leaf C:N ratios, which in turn can affect plant susceptibility to herbivores (Reich et al. 2001). Like autotrophs, interspecific differences in metazoan composition can also be correlated with substrate stoichiometry (Markow et al. 1999, Jaenike and Markow 2003), albeit to a lesser extent. Such patterns could reflect selection for lower nutrient content in the face of limitation. For example, Fagan et al. (2002) found phytophagous insects exhibit lower N concentrations than predators do, and suggested that N scarcity in plant tissue may have selected for lower N dependence in herbivores. Despite these examples, across-taxa comparisons between the stoichiometry of consumers and that of their resources are still rare, especially in animals. Such comparisons should prove important not only for clarifying how nutritional constraints shape the evolution of OS, but also for linking the quality of resources to the biochemistry of consumers that underlies their functional capabilities.

While the elemental composition of consumers may sometimes vary with the stoichiometry of their resources, compositional mismatches are ubiquitous in nature and organisms have numerous adaptations for offsetting this

discrepancy. For instance, plants in N-poor soils may incur substantial C costs to build root nodules to provide substrate for N-fixing bacteria (Raymond et al. 2004). Similarly, insects and vertebrates use multiple pre- and post-ingestive regulatory mechanisms to maintain nutrient balance when diet quality varies (Raubenheimer and Simpson 1997, Frost et al. 2005), including selective foraging for rare resources (Kay 2004), differential assimilation of ingested nutrients (Logan et al. 2004), and selective egestion or excretion of nutrients ingested in excess of demand (Zanotto et al. 1994, Darchambeau et al. 2003). The existence of these mechanisms raises a critical evolutionary question: when and how do organisms develop more efficient mechanisms for obtaining, retaining, and concentrating rare resources, and when does OS – and thus demand – evolve? The answer to this question has important implications for the functioning and evolution of ecological communities.

A serious effort to determine the relative importance and synergistic effects of time horizons, temperature, food quality, and other environmental factors on OS would likely prove to be rewarding. For instance, high temperature may reduce stress brought by nutrient limitation on reaction rates, while P availability may constrain the extent to which organisms can grow rapidly in the face of time constraints. Moreover, identifying molecular and biochemical mechanisms for these adaptations would represent an important contribution to both evolutionary and ecological studies. The extension of ecological tolerance limits often exercised during microevolution is an important mechanism that could be, at least to some extent, governed by stoichiometric flexibility of a species in response to a particular set of environmental factors. These factors would then in principle also influence the kinds of biotic factors that come into play.

## **Biotic interactions**

### **Competition**

Direct competition for limited resources is the biotic interaction that most clearly illustrates the role of stoichiometry in evolution. According to resource competition theory (Tilman 1982, Grover 1997), resource use efficiency and acquisition mechanisms largely determine competitive abilities under constant mortality, and OS likely reflects differences in these traits. Organisms compete for multiple resources and allocate them to different phenotypic functions. Thus, OS may underlie important tradeoffs that determine the outcome of competition, such as the tradeoff between competitive ability and growth rate (Grover 1991, Klausmeier et al. 2004). For example, increased investment in N-rich Rubisco increases capacity for C fixation and growth in autotrophs (Nielsen et al. 1996) but the resulting

increase in N demand may reduce competitive ability when N is limited in the environment. Functional tradeoffs in the ability to compete for multiple resources can facilitate the coexistence of stoichiometrically diverse species, and render the outcome of competition largely unpredictable (Huisman and Weissing 2001). Although tradeoffs related to competitive ability are a dominant theme in animal ecology (Chase et al. 2001), the stoichiometric basis of these tradeoffs remains largely unexplored, especially in terrestrial systems.

Stoichiometric consideration of multiple resource environments is the basis for several recent advances in understanding the evolutionary consequences of competition. For example, simulation models suggest that competition for a mixture of resources leads to adaptive radiation even in spatially homogeneous environments (Chow et al. 2004) and competition for spatially heterogeneous resources may also result in disruptive selection and subsequent diversification of acquisition strategies (Day 2000, Kassen et al. 2000) possibly representing the emergence of stoichiometrically distinct lineages. Interestingly, Pfeiffer and Bonhoeffer (2004) showed that stable polymorphisms may evolve in bacterial populations even when they are initially competing for a single resource. New strains evolve to partially metabolize this single resource and the intermediate metabolites then become distinct resources which other strains may specialize on. Such polymorphisms are evolutionarily stable if the rate of ATP production is maximized and the number of enzymes and the concentration of intermediates in the pathway are minimized. In addition, Pfeiffer et al. (2001) found that high rate/low yield ATP producing bacteria (“fermenters”) may outcompete low rate/high yield ATP producers (“respirators”), especially under high resource supply. They suggest that competition between these alternative strategies of ATP production may have been instrumental in the origin of undifferentiated multicellularity, because cooperation and spatial aggregation may increase the relative success of the respiratory pathway. Although stoichiometric tradeoffs have mainly been implicated in maintaining species coexistence, these studies hint at the important role that the interaction between stoichiometry and competition may play in the evolutionary process.

### **Predation**

A consideration of OS should also lead to a more mechanistic understanding of the evolutionary dynamics of predator–prey interactions. Organismal stoichiometry may reflect the biochemistry and functional capabilities of traits relevant to predators and prey. Moreover, the composition of such traits should also influence their fitness cost. In an energetic or other single currency framework, investment in defensive traits reduces the

resources that are available for growth and reproduction. However, in a stoichiometric framework that considers multiple energy and material currencies, the magnitude and nature of this tradeoff should depend on the compositional similarities of these investments. For example, if defensive and growth-related traits require distinct resources, organisms may face minimal tradeoffs associated with internal allocation but larger acquisition tradeoffs (because accumulation of substrate for defense detracts from gaining material for growth). Moreover, distinct resource requirements entail that the optimal investment in defense will increase when materials for defense are commonly available relative to substrate for growth and reproduction (Kay and Adler 2003).

Ecologists have long recognized that defensive strategies in plants may have stoichiometric underpinnings: one explicitly stoichiometric idea is the carbon-nutrient balance (CNB) hypothesis, which predicts that plants invest more in C- or N-based defensive compounds when either light or nitrogen are particularly abundant in the environment (Bryant et al. 1983). Although the assumptions of the CNB hypothesis have been criticized (Hamilton et al. 2001, Nitao et al. 2002), the influence of the relative availabilities of resources on plant defense (Craine et al. 2003) remains unresolved. Animal stoichiometry may also vary with investment in defensive traits. A variety of animals possess constitutive or induced morphological defenses, such as body armor (Lovegrove 2001), thicker shells (West et al. 1991, Vermeij 1994) and spines (Jeschke and Tollrian 2000). The elemental composition of such mechanical features differs from basic cellular constituents, and could shift OS and resource requirements. Organism stoichiometry could also impinge on predator performance (Fagan et al. 2002). For example, alternative foraging opportunities may favor the evolution of trophic polymorphisms, diversification in growth patterns, and changes in RNA levels (McLaughlin et al. 1999), thus imposing on P requirements. Although animal ecologists have rarely focused on the elemental and biochemical makeup of defensive and offensive requirements, such focus may help to link the nature of defense strategies and the form of predator-prey interactions to the costs of resource acquisition.

The threat of predation may also select for changes in growth rate and other life history traits (Reznick et al. 2001, Altwegg 2002) that may be linked to OS. Foragers can mediate predation risk through vigilance and patch choice (Brown 1999), but each option lowers the potential rates of both food gain and growth. For example, diel vertical migration, which is known to be induced by predation, forces plankton to a colder, nutrient-poor environment during the day, at a substantial energetic cost (Lampert et al. 2003, but see Winder and Spaak 2001). Determining the stoichiometric underpinnings of growth could clarify how resource composi-

tion and other environmental factors modify tradeoffs between safety and food gain, and thereby mediate the form and ecological consequences of non-lethal interactions. Furthermore, predator-induced changes in prey traits may affect how perturbations are transformed in food webs (Werner and Peacor 2003). Analyzing the stoichiometric consequences of such changes may lead to a more mechanistic understanding of trait-mediated interactions, and further integrate evolutionary, community, and ecosystem ecology.

## Mutualism

Organismal stoichiometry may also affect the adaptive consequences of mutualistic interactions. Biological market models suggest that stoichiometry may be a key factor determining the boundaries of mutualism when interactions involve reciprocal exchange of materials (Schwartz and Hoeksema 1998, Sterner and Elser 2002, Hoeksema and Schwartz 2003). These models predict that differences in abilities to acquire distinct resources or in relative demands for those resources will increase the likelihood of mutually beneficial exchange. If these predictions gain empirical support, it would suggest that the presence of stoichiometrically distinct trading partners could allow organisms to colonize new environments, which may in turn increase diversification rate.

Resource exchange mutualisms may also affect the evolutionary dynamics of the resource demands and stoichiometry of interacting species. Some comparisons suggest that trade between facultative mutualists leads to higher tissue concentrations of nutrients received in the exchange (Ning and Cumming 2001, Peterson and Heck 2001). However, little is known about how trade affects the evolution of stoichiometry in obligate mutualists. Recently, molecular approaches have revealed that genomes of obligate symbionts undergo a complex set of modifications that lead to the provisioning of hosts with nutrients that they are unable to synthesize themselves and that are absent or limiting in their diets (Shigenobu et al. 2000, Moran et al. 2003). A possible evolutionary consequence of these associations is a relaxation of the stoichiometric constraints imposed on each species. Indeed, by making certain nutrients more available, trading might relax selection for efficient use of those nutrients. For example, plants with N-fixing bacteria may have intrinsically higher leaf N concentrations (Mattson 1980) and lower N-resorption proficiency than non-fixers (Killingbeck 1996). In contrast, termites, which have N-fixing bacteria in their guts, have N concentrations no higher than is typical for animals, and possess numerous traits for conserving N, such as the production of uric acid instead of urea, ingestion of

exuvia, and cannibalism of nestmates (reviewed by Sterner and Elser 2002).

Mutualisms could also lead to the evolution of growth rate-mediated changes in OS. Nutrient acquisition from facultative mutualists often leads to growth rate increases (Fenchel and Finlay 1991, Peterson and Heck 2001). Similarly, mutualists providing protection may enhance rates of feeding and growth for their partners (Flatt and Weisser 2000). However, exchanging nutrients for protection can also entail physiological costs that reduce growth rate (Stadler and Dixon 1998). Comparing the energetic and elemental requirements of growth to the costs of interactions may lead to a better understanding of the tradeoffs that affect the outcome of facultative mutualisms. More simply, stoichiometric comparisons between individuals in the presence or absence of a facultative mutualist (Kay et al. 2004) may also provide valuable information into the mechanisms and evolutionary consequences of these interactions.

## Sexual interactions

Stoichiometry may also play an important role in sexual interactions, although few studies have considered the adaptive significance of OS in this context. Females and males may differ in elemental composition because of differential investment in primary sexual characters. In some crustaceans, C:P ratios are relatively high in eggs (Sterner and Schulz 1998, Færøvig and Hessen 2003), possibly due to high lipid investment (Tessier et al. 1983), which could enrich females in C. In contrast, *Drosophila* females contain higher P concentrations than conspecific males, perhaps because of the substantial RNA investment for supporting embryogenesis (Markow et al. 1999); this result is consistent with the large amount of rDNA in oocytes of an *Acanthocyclops* copepod (Standiford 1988), and the decrease of RNA:DNA ratios during development of *Daphnia* embryos (Gorokhova and Kyle 2002). Further work is needed to determine the extent of sexual differences in P concentrations across taxa and the physiological and biochemical mechanisms underlying these differences.

Selection for secondary sexual characteristics likely also has far-reaching stoichiometric implications. For example, males in various beetle species possess horns that are often used in fights with other males (Eberhard 1980, Emlen 1997); because insect cuticle contains low % P (Sterner and Elser 2002), larger horns or any other sexually selected exoskeletal outgrowths may significantly increase body N:P ratios. Similarly, selection for enlarged claws in fiddler crabs (Christy and Salmon 1991), or investment in antlers (Moen and Pastor 1998), and other structural weapons and signals likely also has a substantial effect on OS. Focusing on the elemental

requirements of sexually selected traits should help connect the structural properties that affect their function and reproductive value to resource features that constrain their expression. Such work could thus produce significant new insights into the ecological factors that influence the form of sexual interactions and the design of mating systems.

Stoichiometric differences could also result from selection for nuptial gifts transferred by courting male invertebrates. In some cases, nuptial gifts represent a substantial material investment (e.g. up to 40% of a male's body mass in bush crickets, Heller et al. 1998). As a result, compositionally distinct nuptial gifts may skew the stoichiometry and thus resource demands of males. Many studies of nuptial gift composition have focused on N- and C- rich molecules such as amino acids, lipids, and carbohydrates (Wedell 1994), but Markow et al. (2001) found evidence in *Drosophila* that females incorporate P from seminal fluid into ovarian nucleic acids. A focus on the P content of nuptial gifts and the P requirements of reproduction may help to determine in which species and under what conditions such gifts function as parental investment, and when they serve as mating effort.

The alterations in stoichiometric composition due to other courtship-related and mate-seeking traits seem intuitively plausible, but they have not yet received adequate attention. For example, pheromones and color displays can play a decisive role in sexual selection (Andersson 1994). A better understanding of the material demands of these traits could help to reveal the nature of their cost and thus their reliability as indicators of male quality. In addition, more work is needed to determine how production of these compounds affects nutrient balance and species- and sex-specific requirements for elements. Energetic demands also likely impinge on the stoichiometry of sexual behavior. For example, upstream migration in anadromous fish was shown to reduce tissue reserves available for mate competition and development of secondary sexual traits in male chinook salmon (Kinnison et al. 2003). Future work should explore whether the relevance of such energetic tradeoffs can depend on the balance of energy and materials in consumers and their foods.

Thus, although our discussion points to the potential effects sexual selection may have on the evolution of OS, basic information is still needed before the extent of these relationships can be assessed. Discovering the stoichiometric basis of intersexual differences could provide new insight into the form and strength of sexual selection. Elucidating links between stoichiometry and sexual selection may also lead to a more mechanistic understanding of the tradeoffs underlying mating strategies and the evolutionary determinants of mating systems.

Although there has been a great deal of work done on the ecology of biotic interactions, the explicit link between OS, biotic interactions, and fitness remains relatively unexplored. As a result, many exciting hypotheses, such as the link between predation, growth rates, and the evolution of elemental composition, remain to be tested. Future work should also focus on exploring OS in the context of other biotic interactions, such as pathology, cannibalism, etc. that could profoundly impact fitness. Finally, integrating stoichiometric considerations into optimality and adaptive dynamics models (Hofbauer and Sigmund 1998, Clark and Mangel 2000) may provide a powerful approach for evaluating the evolution of ecological interactions.

### Macroevolutionary patterns

Above, we suggested that identifying selective pressures and constraints on OS could help to explain evolutionary patterns. In this section, we discuss how patterns in extant taxa and the paleontological record may help elucidate processes that guide the evolution of OS and the subsequent consequences for ecological interactions.

### Phylogenetic considerations

Several recent studies have used comparative analyses of elemental concentrations to examine patterns of stoichiometric diversity (Cross et al. 2003, Quigg et al. 2003, Wright et al. 2004). Although pattern analysis alone is unlikely to provide a rigorous test of adaptive hypotheses (Reeve and Sherman 2001), comparisons can indicate internal and environmental factors that have influenced phenotypic evolution. To our knowledge, only the studies on insect C:N:P stoichiometry by Fagan et al. (2002) and Woods et al. (2004) have used phylogenetically corrected comparisons (Felsenstein 1985). However, given the recent proliferation of published data on OS (Elser et al. 2000c) and on phylogenies, such comparisons could readily provide important insight into the evolution of OS. For example, OS could be compared to potential selective agents such as nutrient availability and distribution, feeding strategy, predation pressure, and the design of mating systems. Such comparisons could help to explain not only the existence of stoichiometric patterns in the biota, but also the role that OS plays in guiding or constraining the evolution of biotic interactions. In addition, the evolutionary lability of stoichiometry could be compared to that of other behavioral, physiological, or morphological characters. Several studies have suggested that OS has a strong phylogenetic signal (Fagan et al. 2002, Quigg et al. 2003), although the rapidity with which rDNA phenotypes and growth rates can evolve suggests that stoichiometric

traits related to production may be only weakly correlated with phylogeny. Comparing the lability of stoichiometry to that of other traits should help generate important hypotheses pertaining to the causes of character stasis in lineages (see Böhning-Gaese and Oberrath 1999 for the value of comparing the evolutionary lability of traits).

### Temporal patterns

Temporal changes in elemental availability may be intricately linked to the rise and fall of diverse taxa, both in the ocean and on land. In turn, major evolutionary innovations and the associated appearance and diversification of new taxa have profoundly altered key biogeochemical cycles. Changes in the relative abundances of elements appear to have been one of the major determinants of macroevolutionary patterns. For example, Quigg et al. (2003) show that micronutrient stoichiometry of extant marine phytoplankton taxa reflects the composition of their symbiotic plastids. Consequently, historical shifts in the composition of phytoplankton communities may have been driven by changes in trace element solubilities linked to the redox state of the ocean. Elser and colleagues (2003, in press) hypothesize that increases in P availability and a subsequent increase in nutritional quality of stromatogenic autotrophs may have precipitated the “Cambrian explosion” by facilitating the evolution and diversification of multicellular consumers. The unsurpassed sizes of herbivorous dinosaurs may also be related to resource stoichiometry. Burness et al. (2001) argued that large herbivores evolved as a response to high plant productivity under increased CO<sub>2</sub> concentration in the Mesozoic. However, Midgley et al. (2002) suggested that the low quality (high C:N) of plant matter generated by high CO<sub>2</sub> may have favored larger herbivores with longer guts to process forage efficiently. Changes in resource quality may also have played a role in the evolution of insect flight. Hasenfuss (2002) has argued that the appearance of winged insects in the late Devonian (Engel and Grimaldi 2004) concurrently with the first arborescent vascular plants (Bateman et al. 1998) may indicate that the evolutionary migration of high quality plant forage (buds, new leaves) into the canopy favored the emergence of insect flight. Together, these examples suggest that shifts in resource stoichiometry may have generated new ecological opportunities and constraints that had a major impact on the diversification of life.

In turn, evolutionary innovations have modified the stoichiometry of available resources with immense consequences for the evolution of the whole biosphere. For example, the evolution of oxygenic photosynthesis in cyanobacteria dramatically increased oxygen content of the atmosphere (Blankenship and Hartman 1998, Dis-

mukes et al. 2001). Similarly, the appearance of biological nitrogen fixation made fixed nitrogen widely available compared to early Earth conditions (Raymond et al. 2004). Likewise,  $C_4$  photosynthesis evolved in multiple plant lineages ca 30–25 m.y.a., at least in part as a response to low atmospheric  $CO_2$  concentrations that promoted photorespiration (Sage 2004). The global expansion in the late Miocene of  $C_4$  biomass, which generally has high C:N ratios, has been suggested as a factor in the significant turnover of mammalian fauna in the Cenozoic (Cerling et al. 1998). Evolutionary innovations may also have influenced macroevolutionary patterns in distant ecosystems. For example, the coevolution of herbivore size and dentition and silicon (Si)-based structural defenses of grasses (Retallack 2001) may have greatly increased the export of Si to the oceans, which in turn may have contributed to the diversification and ascendancy of marine diatoms (Falkowski et al. 2004). Ecological stoichiometry offers to such macroevolutionary studies an explicit multiple currency capability that can link the interactions between organisms and their environments, including other organisms, to the interacting pools and fluxes of elements and energy.

Stoichiometric considerations may also help reveal changing modes of evolution in patterns of diversification. Using an economic analogy, Vermeij (1994) highlights three pathways to evolutionary intensification that may provide important links between energetic and stoichiometric perspectives: 1) increasing income (faster metabolism), 2) trade (partnerships and interactions), and 3) technological innovation (new chemical pathways or biomechanical structures). Although the first pathway highlights enrichment (i.e. increasing organism size and/or nutrient quotas), the latter two have more complex and interesting stoichiometric implications. To continue the economic analogy, the focus of ecological stoichiometry on the multiple currencies of ecological interactions might be usefully seen as describing the prevailing market conditions under which trade and innovation take place. For example, over the history of terrestrial evolution, Brundrett (2002) has hypothesized that plant–mycorrhizal associations have progressed from ammensal or commensal associations, through obligate, balanced mutualisms, toward exploitive associations benefiting mycotrophic plants at the expense of their fungal partners. This changing trading relationship has entailed an enormous number of biochemical (recognition, defense, digestion) and biomechanical (support, uptake, vascular transport) innovations. Fuller consideration of changing stoichiometric conditions of the geobiosphere may provide a more informative, predictive framework for understanding the energetic and material markets that underlay major macroevolutionary trends.

## Conclusion

We have presented the potential of ecological stoichiometry as a synthetic framework for the study of evolution. Its focus on the material composition of organisms and their resources provides a common measurable currency that is meaningful across scales, linking molecular, ecological, biogeochemical, and evolutionary processes. This material basis also makes explicit feedbacks between the evolutionary diversification of the biota and the environment. Finally, the fact that stoichiometry necessarily entails multiple material and energetic currencies makes it a natural framework for considering evolutionary tradeoffs under diverse selective pressures. The potential utility of ecological stoichiometry for evolutionary biology is illustrated by the diversity of studies and approaches we have collected here, spanning from the molecular to the global.

Throughout this paper, we have highlighted some important open avenues for further research. Promising areas for advancing stoichiometric perspectives in evolution exist at multiple scales and in many disciplines. At the molecular level, genome and proteome studies will be essential for synthesizing knowledge about the mechanistic relationships among hereditary changes, variation in macromolecular composition, phenotypic function, and adaptation across diverse taxa. Concerning microevolutionary dynamics and species interactions, adding stoichiometric currencies to traditional, demographic modeling approaches (e.g. game theory, adaptive dynamics) could lend further insights into evolutionary tradeoffs, adaptive radiation, convergent evolution, and the maintenance of diversity. Further, grounding model parameters in elemental terms should make predictions more amenable to empirical testing. Because traditional evolutionary studies have applied demographic or energetic currencies for evaluating strategies, focusing on materials should also provide new opportunities for exploring how the balance of qualitatively different resources affects the structure of tradeoffs underlying performance. Moreover, integrating this stoichiometric framework with metabolic theory (Brown et al. 2004) could produce a comprehensive organizing concept for evolutionary ecology. At the macroevolutionary level, stoichiometric perspectives can better operationalize models of adaptive escalation and illuminate links between biotic diversification and the development of global biogeochemical cycles. A focus on the stoichiometric ramifications of the major evolutionary transitions could also provide profound insights into the history and functioning of the Earth's biosphere.

*Acknowledgements* – This paper was written as part of Woodstoich: an ecological stoichiometry happening. We thank Jim Elser and Dag Hessen for organizing such a wonderful workshop, and the Centre for Advanced Studies for funding. We appreciate the comments from Jim Elser and Bob Sterner. Thanks to the staff at the FINSE alpine research station. And

finally thanks to Jannicke Moe, for without her logistical support, the workshop would not have been possible.

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