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REVIEW

Biological stoichiometry from genes to ecosystems

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Abstract

Ecological stoichiometry is the study of the balance of multiple chemical elements in ecological interactions. This paper reviews recent findings in this area and seeks to broaden the stoichiometric concept for use in evolutionary studies, in integrating ecological dynamics with cellular and genetic mechanisms, and in developing a unified means for studying diverse organisms in diverse habitats. This broader approach would then be considered "biological stoichiometry". Evidence supporting a hypothesised connection between the C:N:P stoichiometry of an organism and its growth rate (the "growth rate hypothesis") is reviewed. Various data indicate that rapidly growing organisms commonly have low biomass C:P and N:P ratios. Evidence is then discussed suggesting that low C:P and N:P ratios in rapidly growing organisms reflect increased allocation to P-rich ribosomal RNA (rRNA), as rapid protein synthesis by ribosomes is required to support fast growth. Indeed, diverse organisms (bacteria, copepods, fishes, others) exhibit increased RNA levels when growing actively. This implies that evolutionary processes that generate, directly or indirectly, variation in a major life history trait (specific growth rate) have consequences for ecological dynamics due to their effects on organismal elemental composition. Genetic mechanisms by which organisms generate high RNA, high growth rate phenotypes are discussed next, focusing on the structure and organisation of the ribosomal RNA genes (the "rDNA"). In particular, published studies of a variety of taxa suggest an association between growth rate and variation in the length and content of the intergenic spacer (IGS) region of the rDNA tandem repeat unit. In particular, under conditions favouring increased growth or yield, the number of repeat units ("enhancers") increases (and the IGS increases in length), and transcription rates of rRNA increase. In addition, there is evidence in the literature that increased numbers of copies of rDNA genes are associated with increased growth and production. Thus, a combination of genetic mechanisms may be responsible for establishing the growth potential, and thus the RNA allocation and C:N:P composition, of an organism. Furthermore, various processes, during both sexual and asexual reproduction, can generate variation in the rDNA to provide the raw material for selection and to generate ecologically significant variation in C:N:P stoichiometry. This leads us to hypothesize that the continuous generation of such variation may also play a role in how species interactions develop in ecosystems under different conditions of energy input and nutrient supply.

Keywords

Food webs, growth rate, herbivores, life history evolution, phosphorus, rDNA, RNA, stoichiometry.

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INTRODUCTION

During this century, biology has become increasingly sophisticated and predictive. With increasing sophistication has come increasing specialisation (Maienschein 1991). Narrowing of research focus occurs not only at particular levels of organisation (e.g. ecosystem science, molecular genetics) but also within the confines of particular model organisms (e.g. *Drosophila*, *Arabidopsis*) or of particular habitats (e.g. lakes, temperate forests). As a result, knowledge is increasingly fragmented and it has become more difficult to connect disparate areas of

inquiry in order to appreciate the full context of living things as functional wholes (Appel 1988; Allen & Hoekstra 1992; Pickett *et al.* 1994; Smocovitis 1996; Vogel 1998). Biologists now face the challenge of developing broadly generalisable approaches for the study of living systems that can re-connect our deepening understanding of individual levels of biological organisation and highlight the concurrence of phenomena across diverse biota and ecosystems.

Integrating our growing knowledge base across multiple levels of organisation, diverse types of organisms and contrasting habitats is a daunting challenge. Is it possible that coherent hypotheses can be posed to link the disparate levels of organisation under study by modern biology, from the molecular structure of genes to ecosystem dynamics? We believe that a rich suite of principles emerges when one couples the first law of thermodynamics, the principle of evolution by natural selection and the central dogma of molecular biology. We call this approach "biological stoichiometry". In this paper we describe recent developments in this area and suggest how biological stoichiometry may someday be able to successfully integrate from genes to ecosystems, from microbes to metazoans and from oceans to deserts.

BIOLOGICAL STOICHIOMETRY – WHAT IS IT AND WHERE DID IT COME FROM?

Biological stoichiometry is the study of the balance of energy and multiple chemical elements in living systems (Reiners 1986; Sterner 1995; Elser *et al.* 1996; Hessen 1997). It has its roots in the work of A. J. Lotka (1925), one of the first to consider how thermodynamic laws of physical–chemical systems structure the living world. Lotka's thinking echoes in concepts that are now cornerstones of ecology: optimal foraging (Belovsky 1978), resource ratio competition theory (Tilman 1982), the Redfield ratio in oceanic biogeochemical cycling (Redfield 1958) and nutrient use efficiency (Vitousek 1982).

A direct application of Lotka's contributions can be seen in how stoichiometry mediates food web dynamics. Disparities in elemental composition between animals and their food can affect animal feeding behaviour (Butler *et al.* 1989; White 1993), consumer population stability (White 1993; Sterner & Hessen 1994; Andersen 1997) and community organisation (White 1993; Gulati *et al.* 1991; Andersen 1997; Fig. 1d). Stoichiometric balance also impinges on trophic dynamics (Urabe & Sterner 1996; Sterner *et al.* 1997; Elser *et al.* 1998; Sterner *et al.* 1998)

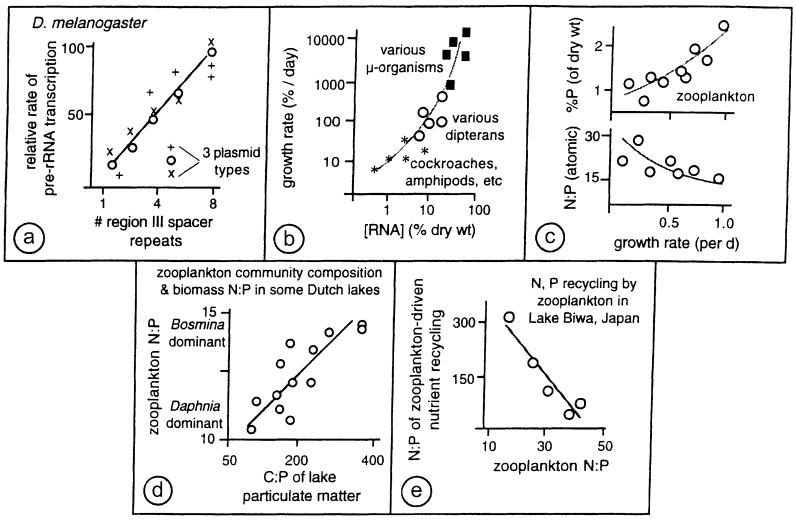

Figure 1 Hypothesised chain of causality linking the molecular genetics of growth to ecological consequences via C:N:P stoichiometry. Direct or indirect natural selection on growth rate generates genetic change associated with ribosomal genes [for example, differences in the number of repeat units in the IGS of the rDNA may result in increased production of rRNA, as shown in (a); modified from Grimaldi & Di Nocera (1988)] because high RNA content is needed for rapid growth [(b); modified from (Sutcliffe 1970)]. Thus, there is an association between growth rate and organism C:N:P because RNA is P-rich [for example, in crustacean zooplankton, rapidly growing animals have high P-content and low N:P, as shown in (c); modified from (Main *et al.* 1997)]. In ecosystems, food with unbalanced elemental composition can influence the community dominance of consumer taxa with different characteristic C:N:P [for example, in lakes with P-rich seston at the base of the food web, P-rich, low N:P *Daphnia* dominate the zooplankton community but in lakes with P-deficient seston, low P, high N:P animals like *Bosmina* are the dominants; (d), modified from (DeMott & Gulati 1999)]. Furthermore, differences in the somatic C:N:P of consumers can affect nutrient excretion rates and ratios in the internal nutrient cycle of ecosystems as grazers attempt to maintain physiological homeostasis [for example, in nutrient recycling studies in Lake Biwa, Japan, high recycling N:P ratios occurred when the zooplankton community was dominated by low N:P taxa but recycling N:P was low when high N:P taxa predominated; (e), modified from (Urabe *et al.* 1995)].

and biogeochemical cycling in food webs (Andersen 1997; Sterner *et al.* 1992; Elser & Hassett 1994; Urabe *et al.* 1995; Schindler & Eby 1997; Vanni & Layne 1997; Elser & Urabe 1999; Elser *et al.* 2000d; Fig. 1e). Direct evidence now exists for direct P-limitation of *Daphnia* growth when feeding on algae of high C:P ratio, both from laboratory studies (Urabe *et al.* 1997) and field experiments (Elser *et al.* 2000c). A key insight from this work is that organisms that must maintain elevated body nutrient content (e.g. low body C:P ratios) are generally more susceptible to poor food quality when available food develops increased C:nutrient ratios (Sterner & Schulz 1998). More recently, studies have begun to highlight how such stoichiometric constraints may shape animal evolution (Elser *et al.* 1996, 2000a; Markow *et al.* 1999; Moen *et al.* 1999). Furthermore, stoichiometric effects have also been demonstrated for microbial food webs in aquatic and terrestrial realms, mediating the tendency for net mineralisation vs. immobilisation of nutrients (Tezuka 1989; Chrzanowski & Kyle 1996; Paul & Clark 1996) and flagellate–bacteria interactions (Caron *et al.* 1985, 1990; Vadstein *et al.* 1993; Nakano 1994). Thus, C:N:P balance has proven to be a powerful tool for analysing ecological systems.

But what determines the C:N:P stoichiometry of living biomass? We and colleagues have proposed that, in organisms lacking major mineral storage of P (as in vacuoles or in bones), biomass C:N:P depends largely on the disproportionate demands for P-rich ribosomal RNA (rRNA) in rapidly growing cells (Fig. 1b; Elser *et al.* 1996; Hessen & Lyche 1991). This *growth rate hypothesis* potentially connects cellular allocation to organism function and ultimately to ecological dynamics. In this paper we extend this hypothesis to the level of molecular genetics. To do so, we first synthesise information regarding the connection between growth rate and C:N:P stoichiometry and then consider pertinent studies that connect biomass C:N:P to rRNA allocation and ultimately to the organisation of ribosomal genes in diverse biota. Our synthesis suggests a thread of causality linking the evolutionary genetics of rRNA to biogeochemical cycling and trophic dynamics in ecosystems.

THE ASSOCIATION BETWEEN C:N:P STOICHIOMETRY AND GROWTH

Growth is linked directly to fitness, as organisms must grow to reproduce. While life history strategies involve a rich suite of characters generated by multifaceted selective pressures (Stearns 1992), Arendt (1997) has argued that specific growth rate is a central integrating parameter of overall life history strategy. (Specific growth rate is the rate of change in mass per unit mass; hereafter, μ. To clarify discussions in the following, let μ_m designate the maximum μ that a particular species at a particular life stage can achieve at a standard temperature. We assume that μ_m is a genetically determined trait characteristic of a particular species' developmental program. Furthermore let μ' be the realised μ at a given time; μ' may be reduced below μ_m by resource conditions.) Arendt (1997) also suggests that a relatively unexplored set of tradeoffs associated with rapid growth life-styles (high μ_m) is likely. We suspect that some of these tradeoffs reflect the stringent stoichiometric demands of rapid growth (Sterner & Schulz 1998; Elser *et al.* 2000a). Indeed, if there are clear connections between C:N:P and μ_m, then there also would be connections between life history evolution, physiological demand for major elements, and even ecosystem processes. What evidence is there for relationships between C:N:P and μ?

Autotrophs are characterised by wildly variable C:N:P composition. This variation has both interspecific and intraspecific components. Across a wide range of autotrophs from algae to trees to cactus (Nielsen *et al.* 1996), percentage N and percentage P of photosynthetic biomass decrease with increasing organism size, a change accompanied by decreasing μ_m. Closer analysis of these trends shows that, as μ_m increases, percentage P increases faster than percentage N and thus rapidly growing, small autotrophs have low N:P. Thus, at the interspecific level, there is major variation in C:N:P coupled to allometry and μ_m. Much variation in C:N:P in autotrophs also occurs at the physiological level as a result of plants' abilities to store energy and nutrients in excess of immediate demands and, in a strategy of unbalanced growth, to flexibly adjust their growth rate (μ') to ambient conditions (Chapin 1980; Marschner 1995). Indeed, when grown under wide-ranging conditions, one species of algae can exhibit nearly as much variation in C:N:P as exists in the data set studied by Nielsen and colleagues (Nielsen *et al.* 1996). Rules of thumb for autotroph C:N:P are: biomass N:P tracks N:P of the nutrient supply (Rhee 1978); at fixed supply rate of nutrient X, biomass C:X increases as light intensity and/or pCO_2 increase (Greenwood 1976; Rhee & Gotham 1981; Smith 1983; Cotrufo *et al.* 1998); and under conditions of X-limited growth, biomass C:X increases steeply as μ' declines (Goldman *et al.* 1979; Droop 1983; Agren 1988). The picture that emerges is one of enormous variation in C:N:P at the base of food webs (Elser *et al.* 2000b).

In contrast to the extreme plasticity in C:N:P exhibited by autotrophs in laboratory cultures, metazoans of a given life stage undergo balanced growth and appear to regulate elemental composition within narrower bounds, even when consuming food with large variation in C:N:P composition (Andersen & Hessen 1991; Sterner & Hessen 1994). Note, however, that physiological accumulation of large energy storage compounds (fats, wax esters and

lipids lacking in N and P) can cause temporal variation in biomass C:nutrient ratios in animals with such storage capabilities. In general, however, most animals maintain a relatively tight homeostasis in C:N:P stoichiometry. When nutrient element X is in excess relative to requirements, consumers increase the specific rate of loss of that element (via excretion or egestion; Urabe 1993; DeMott *et al.* 1998). When challenged with food of low nutrient content (high C:X relative to their body), animals can suffer a growth penalty (μ' declines) and their biomass C:X increases only moderately (DeMott *et al.* 1998). In contrast to the muted plasticity in metazoan C:N:P within a species, there is considerable (previously unappreciated) variation in biomass C:N:P stoichiometry among different taxa, even within taxonomic orders. Crustacean zooplankton alone vary more than five-fold in body P content (Fig. 1c), ranging from $\sim$ 0.5% P in adult copepods to $\sim$ 2.6% P in particular anomopods (Andersen & Hessen 1991; Hessen & Lyche 1991; Main *et al.* 1997). Herbivorous insects in terrestrial systems exhibit a similar range of variation in N and P content (Elser *et al.* 2000b). In addition, variation in zooplankton C:N:P stoichiometry seems associated with μ_m: in the study of Main and colleagues (Main *et al.* 1997), rapidly growing taxa and/or life stages had slightly elevated percentage N but much more strongly increased percentage P and, as a result, had low body N:P. In a comparative study of taxa within the *Daphnia pulex* species complex, Elser *et al.* (2000a) showed that, relative to individuals from temperate habitats, individuals from the arctic (where growing season is short and selection on growth rate should be severe) had higher μ_m and body percentage P, were inefficient recyclers of P, and were more susceptible to P-deficient diets. In both these zooplankton studies we see a striking association between growth and P metabolism.

It is important to note that this paradigm of nutrient composition has primarily been developed in study of freshwater zooplankton. The degree of regulation of element content, its allometric and taxonomic correlates, and the coupling of μ and C:N:P stoichiometry in other groups of organisms is still very poorly known. Is there any reason to expect that similar patterns should hold for other groups of organisms? Concordant patterns would be expected if the patterns observed in zooplankton are associated with fundamental cellular mechanisms shared with other biota. To determine if this is the case, we need to consider potential underlying mechanisms driving the association of C:N:P stoichiometry with growth rate.

THE CELL BIOLOGY OF ORGANISMAL C:N:P-GROWTH STOICHIOMETRY

Although an empirical relationship between growth and C:N:P is clear, the mechanisms underlying the relationship are not. Why do rapidly growing organisms have such high P content and thus low biomass C:P and N:P? Several results implicate growth rate and variation in cellular allocation to P-rich ribosomal RNA (rRNA) as the source of variation in μ_m among organisms (Fig. 1c). For example, in metazoan zooplankton, low-P ($\sim$ 0.5% P), high-N:P calanoid copepods have only $\sim$ 2% RNA by weight while P-rich ($\sim$ 1.3% P), low-N:P *Daphnia* have $\sim$ 10% RNA (Båmstedt 1986; McKee & Knowles 1987). Because RNA is $\sim$ 10% P by weight, differences in RNA-P are entirely sufficient to explain the difference between *Daphnia* and adult copepods in whole organism P content (Sterner 1995; Elser *et al.* 1996). This difference is consistent with the known life histories of these taxa: calanoid copepods are long-lived, slow-growing as adults with relatively low reproductive output whereas *Daphnia* are fast-growing, generalists that produce multiple cohorts of large offspring. This association between growth and RNA concentration appears to be broadly applicable across diverse organisms (Fig. 1b). (Let $[RNA]'$ be the instantaneous RNA concentration of an organism at a given time and $[RNA]_m$ be the maximum [RNA] characteristic of a particular species at a particular life stage.) From *Salmonella* to cockroaches, organisms with high μ_m have high $[RNA]_m$ (Sutcliffe 1970). This makes perfect sense as: (1) RNA makes up 50–60% of the ribosome, the machine of growth in the cell (Becker 1986); (2) the steady-state level of this ribosomal RNA (rRNA) usually comprises 80–90% of the total cellular RNA, with increasing total allocation at high growth rate (Alberts *et al.* 1983) and (3) approximately 10 million ribosomes are necessary to support protein synthesis in the average cell (Lewin 1980).

These studies lead us to suggest that a P-rich, low-N:P signature of rapid growth is *a cellular necessity* derived from the fact that ribosomes are unusually P-rich (Elser *et al.* 1996). We would expect, then, a consistent positive association between μ_m and $[RNA]_m$, while biomass C:P and N:P should generally decline with μ_m. Indeed, this appears to be the pattern in crustacean zooplankton (Fig. 1c), and it is commonplace in cell culture, aquaculture and fisheries studies to use $[RNA]'$, RNA′:DNA, or RNA′:-protein to assess the growth status (μ') of target organisms, including fish (Bulow 1987; Buckley & Caldarone 1999), decapods (Juinio & Cobb 1994), mysids (Båmsted 1983), copepods (Ota & Landry 1984; Saiz *et al.* 1998; Wagner *et al.* 1998), *Daphnia* (McKee & Knowles 1987; Barber *et al.* 1994), chaetognaths (Båmsted 1983), phytoplankton (Rhee 1973; Dortch *et al.* 1983) and bacteria (Kato 1994; Massana *et al.* 1998). Furthermore, changes in $[RNA]_m$ during ontogenetic development of a single species [e.g. in copepods (Båmsted & Skjoldal 1976), euphausids (Skjoldal & Båmsted 1976), herring

larvae (Clemmesen 1994)] and in [RNA]′ in response to environmental variability [e.g. in temperature (Saiz *et al.* 1998), in food availability (Malloy & Targett 1994; Wagner *et al.* 1998)] have been documented. It is interesting to note that measurements of insect RNA levels under field conditions seem almost completely lacking. Nevertheless, there seems ample evidence in existing studies that high growth rate lifestyles are associated with elevated RNA allocation. But how is a high RNA phenotype generated? What are the genetic mechanisms necessary to achieve elevated cellular rRNA and thus high μ_m?

RIBOSOMAL RNA GENES: FUNCTIONAL VARIABILITY IN COPY NUMBER AND IN THE INTERGENIC SPACER?

To answer these questions, one needs to know the molecular organisation of ribosomal RNA genes and associated spacer regions in the genome (rDNA), its variability and the functional significance of this variation in satisfying the demand for rRNA in different organisms. In most eukaryotes, rRNA genes are present in high copy number and are clustered as tandem repeats at one or more chromosomal sites, termed nucleolus organiser regions. Each rDNA array consists of a transcription unit, which includes the genes for the 18S, 5.8S and 28S rRNA subunits (the "*rrn*" genes), the internal transcribed spacers separating the genes within a unit, and an intergenic spacer (IGS, formerly NTS – nontranscribed spacer) separating adjoining units. Because of their structural and catalytic functions during protein synthesis, the *rrn* genes are essential for the support of cell differentiation, development, and growth. That is why they are usually present in high copy number (see reviews by Long & Dawid 1980; Cortadas & Pavon 1982; Sollner-Webb & Tower 1986). Thus, rDNA array size is a function of copy number and gene/spacer repeat length. The transcription unit as a whole is usually highly conserved across a wide range of species and shows a high degree of sequence and secondary structure similarity among phylogenetically diverse taxa (Goldman *et al.* 1983; Elwood *et al.* 1985). In contrast, the subrepetitive elements within the IGS appear to be changing rapidly and, as a result, IGS regions can vary not only between different species but also between populations, individuals, and even within a single cell (Boseley *et al.* 1979; Paule & Lofquist 1996; Ganley & Scott 1998). Both restriction-site variation and, more commonly, length variation occur (Rogers & Bendich 1987). It is now known from molecular genetic studies that the IGS is critically important for the regulation of rDNA metabolism, carrying all the elements necessary for transcription and correct termination of rRNAs (for references see Reeder & Dunaway 1983). Indeed, Polymerase I transcribes through the IGS of *Xenopus* (Labhart & Reeder 1987) and *Drosophila* (Grimaldi *et al.* 1990) and promoter-proximal termination IGS sequences stimulate initiation of transcription in vertebrates (Grummt *et al.* 1986; Henderson & Sollner-Webb 1986). Given these regulatory properties, variation in sequence and organisation of the IGS seems highly functional, resulting in "molecular coevolution" (Dover 1982) of the transcription machinery (Evers & Grummt 1995).

Despite the apparent functional significance of rDNA variation suggested by molecular studies and the solid evidence for variation of rDNA structure in different taxa, to date rDNA heterogeneity has largely been studied for construction of phylogenetic relationships and for quantification of gene flow between populations (Gray & Schnare 1996). There is little knowledge concerning the effects of rDNA heterogeneity on growth and development, performance of different organismal functions, and adaptive response; needless to say, the consequences for ecosystem dynamics owing to such variation have not yet been contemplated. However, several lines of evidence indicate that variation in the intergenic spacers has major functional consequences due to the relationship between cellular RNA levels and organism growth rate (Table 1). For example, under selection for development time (μ_m), spacer length in *Drosophila melanogaster* increased in concert with accelerated development, i.e. increased μ_m (Cluster *et al.* 1987). This response is consistent with the idea that, as for other genes, transcription rates of rDNA genes are increased by the presence of enhancer regions (Paule & Lofquist 1996). In the case of rDNA, these enhancers are located in the IGS and occur in multiple copies that vary both intra- and interspecifically (Reeder *et al.* 1983; Paule & Lofquist 1996). Indeed, another study of *D. melanogaster* has shown that the rate of transcriptional production of prerRNA is directly proportional to the number of enhancers in the IGS (Fig. 1a, Table 1; Grimaldi & Di Nocera 1988). This suggests that there should be relationships between the multiplicity of enhancers in the IGS (and hence variation in spacer length), $[RNA]_m$, and μ_m. Increased numbers of rDNA enhancers should act to increase the rate of rRNA production by RNA Polymerase I. However, rates of Polymerase I transcription per rDNA gene have an upper limit and one might then expect, under strong selective pressures for high μ_m, increased dosage of rDNA genes in order to further increase rRNA production rate. Indeed, it appears that organisms have adopted diverse strategies to assure sufficient rRNA production according to the particular demands of their life history (Table 2). The tendency to increase copy number of ribosomal genes is

Table 1 Observations related to variation in the structure of rDNA genes (as *rrn* copy number, IGS length) and growth-or rRNA-related parameters in different species

Organism	Observations	Reference
Ribosomal DNA copy number		
Flax	Levels of rDNA at the apex (fast growing cells) changed in response to the growth rate and nutrient regime whereas rDNA at the base of the stem (slow growing cells) remained constant.	Cullis & Charlton (1981)
Soybean cell cultures	Reduction in rDNA (~30%) was concomitant with reduction in growth rate caused by poor nutrient supply	Jackson (1980)
Fruit fly (*Drosophila melanogaster*, *D. hydei, D. mercatorum*)	Strains containing 30–100 copies have slower development compared to those with 100 copies or more; mutant individuals carrying a reduced number of *rrn* genes exhibit a bobbed phenotype, characterized by a retarded development, reduced growth, a thinner cuticle, smaller bristles, etc.	Shermoen & Kiefer (1975); Ritossa (1976); Franz & Kunz (1981); Templeton *et al.* (1985); Desalle *et al.* (1986)
Amphibian oocytes	Temporary amplification of rRNA genes as an adaptation to large cell volume and increased need for rRNA synthesis.	Gall (1968)
Chicken	Increased rDNA gene dosage and larger nucleoli in chickens selected for rapid growth.	Delany *et al.* (1994a)
Chick embryo	Lower cellular RNA levels and reduced development in embryos with deficiency for *rrn* gene copy number.	Delany *et al.* (1994b)
IGS length variation		
Maize (*Zea mays*)	Frequency of long-spacer variant higher, and frequency of short-spacer variant lower, in maize strains selected for increased yield.	Rocheford *et al.* (1990)
Wheat (*Triticum dicoccoides*)	Spacer diversities in natural populations were significantly correlated and predictable in terms of climatic variables.	Flavell *et al.* (1986)
Oats (*Avena sativa*)	Bred cultivars had significantly longer IGS regions than landraces.	Polanco & Perez de la Vega (1997)
Fruit fly (*Drosophila melanogaster*)	Under selection for development time, spacer length increased in concert with shortened development time; rate of transcriptional production of prerRNA was directly proportional to the number of enhancers located in the IGS.	Grimaldi & Di Nocera (1988); Cluster *et al.* (1987)
Xenopus oocytes	In an *in vitro* transcription assay, longer IGS's were more competitive than shorter IGS's in binding Pol 1	Reeder *et al.* 1983
Chicken	Selection for reproductive (egg layers) and somatic (broilers) growth resulted in specific variability in two different regions of IGS.	Delany & Krupkin (1999)

rather obvious. While ribosomal genes can be amplified externally in certain organisms and tissue types (e.g. oocytes), most variation occurs in chromosomal rDNA copy number. According to the review of Long and Dawid (Long & Dawid 1980), rDNA gene copy number within the Class Insecta alone varies at least 7-fold (and at least 3.5-fold within the drosophilids). In plants at least, it appears that many of the added copies of rDNA genes are superfluous (Rogers & Bendich 1987); thus, to evaluate these ideas it will be necessary to distinguish between active and inactive rDNA genes in the genome. In any case, while many authors have implicated ecogeographic factors connected to genetic variation in rDNA in particular taxa, to our knowledge no studies have examined the functional significance and evolutionary ecological implications of rDNA copy number and spacer length variation across a wide range of taxa and selective environments. We hypothesise that major features of the organisation of rDNA genes (such as spacer length, copy number) underpin phenotypic variation in rRNA synthesis, ribosomal production and thus also μ_m, $[RNA]_m$ and C:N:P. Furthermore, if C:N:P variation has broad ecological significance as indicated by various recent studies, particular behavioural, physiological and ecological correlates should accompany major patterns in the structure of rDNA genes.

Observation of significant variation in the organisation of the rDNA genome raises the question of how such variation is generated at the molecular level. Indeed, the fact that rDNA copy number is known to vary so widely

Table 2 Genetic mechanisms by which a large demand for rRNA can be satisfied in different organisms

Way to increase rRNA synthesis	Organism	Reference
Transcription rate per gene varies to match the growth rate.	bacteria	Nomura *et al.* (1984)
Extra-chromosomal amplification: one to several copies of rDNA are used as templates to produce hundreds to thousands of extra-chromosomal copies. Usually this event only occurs at specific stages; however, in some species rRNA genes may be entirely extrachromosomal.	protozoans	Kafatos *et al.* (1985)
	slime moulds	Vogt & Braun (1976); Vogt & Braun (1977); Lewin 1980, Long & Dawid 1980; Welker *et al.* (1985); Williams (1986)
	some animal oocytes	Thiebaud (1979); Nomura *et al.* (1984); John & Miklos (1988)
Replication of the entire genome: polyploids are created through rounds of DNA replication without cell division.	many plants	Rogers & Bendich 1987
Large quantities of highly repetitive rDNA are present in nucleoli of oocytes and then diminuted, leaving somatic cells with low number of copies but oocyte rich in rRNA.	some cyclopoid copepods	Standiford (1988)
Each nucleus contains a large number of rRNA genes, 100–1000 copies per diploid cell in animals and 500–40 000 copies in plants.	most eukaryotes	Rogers & Bendich (1987); John & Miklos (1988)

within a species suggests that duplication and deletion of repeats is easy and frequent. Divergence of rDNA is directly tied to the same forces that influence variation of any repetitive DNA: mutation, unequal crossing-over, nonhomologous exchange, intrachromosomal recombination, gene conversion, and random drift (Ohta & Dover 1983; Ganley & Scott 1998). The relative roles of these processes in inducing and maintaining rDNA variability are uncertain, although it is believed that some of these mechanisms are linked (Szostak *et al.* 1983). Intrachromosomal recombination (unequal crossing-over or gene conversion between sister chromatids) is probably the most important (e.g. Schlötterer & Tautz 1994) but interchromosomal exchange can also take place at high rates in *Drosophila* (Polanco *et al.* 1998). Fluctuations in either rDNA copy number and/or IGS length are thought to occur by unequal crossing over at the level of the entire rDNA units or at the level of IGS subrepeats, respectively (Dover *et al.* 1993). Recombination can be mitotic or meiotic and therefore repeat variants can arise during both sexual and asexual reproduction or even in somatic cell lineages (Cullis & Charlton 1981). For example, it has been shown recently that unequal crossing over in the fungus *Neotyphodium* can produce significant changes in the IGS in just two generations (Ganley & Scott 1998). Thus, the overall picture with respect to genetic mechanisms is that there are many means by which major changes in the organisation of the IGS can be generated during production of gametes, mitotic production of asexual propagules, and even during mitotic production of somatic cells. If, as suggested by material reviewed in preceding paragraphs, such variations have functional significance, then considerable raw material for the evolution of C:N:P – growth stoichiometry is constantly being made available. Whether these connections to evolution and genetics have major effects on the structure and function of food webs remains to be seen. We suspect that they do and conclude by proposing that it may now be possible to generate a functionally realistic model of ecological dynamics informed by modern genetic understanding.

THE MOLECULAR GENETICS OF FOOD-WEB DYNAMICS: A HYPOTHESIS

The work we have described indicates that evolution of growth rate and molecular mechanisms related to RNA allocation are inextricably linked. Furthermore, it seems clear that growth and C:N:P stoichiometry of organisms are closely connected due to major effects of RNA allocation on organism P-content. Finally, major effects of organism C:N:P stoichiometry for trophic dynamics, population stability and nutrient cycling are manifest from a variety of recent findings. This synthesis leads us to hypothesise that patterns of community structure, trophic interactions, and biogeochemical cycling in food webs are generated by specific molecular genetic variations among interacting and coevolving biota. In the most general sense we predict that variation in the relative abundance of high growth rate, low C:P and N:P consumer taxa with relatively long IGS regions and elevated rDNA dosage should be higher in systems with abundant, good quality (low C:N and C:P) food. Conversely, only slow growing taxa (with high C:P and N:P ratios, short IGS regions, and low rDNA copy

number) should be able to persist in strongly nutrient-deficient systems with poor quality (high C:P and C:N) food. Because the mechanisms described are fundamental, we expect that these patterns should hold both within particular habitats (e.g. across different lakes differing in trophic status, across agricultural fields subjected to different levels of fertilisation) as well as across habitats (e.g. oceans to lakes to wetlands to deserts). Furthermore, reflecting the consequences of stoichiometric constraints for consumer dynamics (Andersen 1997), we also expect that consumers with elevated RNA allocations due to variation in their ribosomal genome will enjoy a growth advantage during good food conditions but will be unusually susceptible to stoichiometrically unbalanced food. As a result, they may have particularly erratic population dynamics and be more vulnerable to deterministic extinction (Andersen 1997).

Furthermore, an appreciation of the molecular mechanisms by which functionally significant variation in key rDNA genes is generated and of the functional consequences of that variation permits us to hypothesise how stoichiometric relations might operate in the self-organisation of trophic interactions and nutrient cycling in food webs over evolutionary time. Imagine a limited number of individuals in a few functional groups (e.g. primary producers, herbivores, detritivores, decomposer bacteria, higher predators) introduced to an environment with a set supply of chemical elements and external solar energy inputs. Upon introduction, some set of interactions will inevitably proceed and indeed such dynamics are the topic of most existing food web models. However, it no longer seems prudent to ignore evolutionary change in consideration of ecological dynamics (Thompson 1999), especially given the apparent propensity of ribosomal genomes to reorganise with significant consequences for organism life history and physiological function. Thus, if the growth-related genes are allowed to mutate (with their associated effects on organism C:N:P stoichiometry), one can imagine that some environments (low phosphorus vs. high phosphorus, for example) might favour or disfavour particular strains, with concurrent effects on the web of trophic interactions and recycling feedback. It is not clear from any existing theory what shape such food webs should eventually develop. We suggest that recent increases in our understanding of these stoichiometric relationships now make it feasible for such a theory to be constructed and tested.

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BIOSKETCH

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