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THE FUNCTIONAL SIGNIFICANCE OF RIBOSOMAL (r)DNA VARIATION: Impacts on the Evolutionary Ecology of Organisms

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■ **Abstract** The multi-gene family that encodes ribosomal RNA (the rDNA) has been the subject of numerous review articles examining its structure and function, as well as its use as a molecular systematic marker. The purpose of this review is to integrate information about structural and functional aspects of rDNA that impact the ecology and evolution of organisms. We examine current understanding of the impact of length heterogeneity and copy number in the rDNA on fitness and the evolutionary ecology of organisms. We also examine the role that elemental ratios (biological stoichiometry) play in mediating the impact of rDNA variation in natural populations and ecosystems. The body of work examined suggests that there are strong reciprocal feedbacks between rDNA and the ecology of all organisms, from microbes to metazoans, mediated through increased phosphorus demand in organisms with high rRNA content.

INTRODUCTION

The increasingly integrative nature of science in the twentyfirst century has opened up fresh vistas in the fields of ecology, evolutionary biology, and molecular genetics, whereby a synthesis of techniques and perspectives has provided new advancements in these fields (e.g., Feder & Mitchell-Olds 2003). Herein we examine

a central cellular component, the ribosome, and describe how the multi-gene family encoding ribosomal(r)RNA (the rDNA) plays a major functional role in the evolutionary ecology of organisms. Past reviews have examined the basic structure and cellular function of rDNA/rRNA (for example, Flavell 1986, Fromont-Racine et al. 2003, Moore & Steitz 2002, Sollner-Webb & Tower 1986), the evolution of rDNA among organisms (Gerbi 1985), and the use of rDNA as a molecular marker for systematics and phylogenetic reconstruction (Hillis & Dixon 1991, Mindell & Honeycutt 1990). We address the interaction between rDNA and important life-history features, especially growth rate, and how selection on rDNA variants plays an important role in the microevolutionary processes in many natural populations. We conclude by applying the emerging perspective of biological stoichiometry (Elser et al. 2000b, Sterner & Elser 2002) to examine how evolutionary forces operating on variation in rDNA can have a major effect on ecological interactions, such as competition, trophic production, and biogeochemical cycling through the central role played by ribosome biogenesis in determining growth and resource requirements.

BACKGROUND

Why study rDNA variation? Our basic thesis is that ribosome biogenesis is one of the most central processes in cellular biology from a functional perspective because of its close connections to the pace of growth and development [e.g., growth regulation mechanisms such as stringent control versus growth-related control in prokaryotes (Gourse et al. 1996), as rDNA transcription is the first step of ribosome biogenesis (Sollner-Webb & Tower 1986)]. Furthermore, rRNA synthesis represents a large energetic and nutrient sink for all growing organisms, representing ~80% of cellular RNA content and 20% of total cell dry weight (Neidhardt et al. 1990). Much work in this area has focused on well-studied prokaryotes (i.e., relationship between rDNA structure and regulation and organismal function in *Escherichia coli*), but the full range of connections between rDNA structural variation and variation in rDNA expression is not completely appreciated by many researchers who study eukaryotes. In addition, the ramifications of rRNA metabolism are likely to range far beyond cell biology and will impact all organismal functions related to growth rate (e.g., life-history evolution), as well as represent a fundamental component of ecological production (Sterner & Elser 2002).

We first review the basic structure of rDNA in prokaryotes and eukaryotes; describe what is known about how transcriptional efficiencies of the rRNA-encoding genes can be influenced by variations in copy number (CN), spacer length variation, regulatory regions, and epigenetic effects; and discuss how these variations may influence growth rate. We then examine evidence for either artificial or natural selection on rDNA variants across a range of organisms. Furthermore, we will show how rDNA variations can be directly linked to the ecology of an organism via rDNA growth connections and impacts on production. Finally, because rRNA

represents a critical pool of phosphorus (P) in many organisms, we link rDNA variations to the nutrient requirements of organisms, focusing on carbon:phosphorus (C:P) stoichiometry, and to the impacts of biogeochemical cycling of P as a key limiting nutrient influencing ecosystem functions.

Basic Structure of rDNA

Ribosomes are the sites of protein synthesis in all organisms. These organelles basically consist of two subunits (the large and small subunits) composed of ribosomal RNA (rRNA) and proteins and whose size (molecular weight) varies depending on systematic affinity. In prokaryotes (i.e., *E. coli*), the ribosome consists of a *30S* (small) and *50S* (large) subunit that make up the functional (*70S*) ribosome. The small subunit contains a *16S* rRNA molecule, whereas the large subunit contains a *5S* and a *23S* rRNA molecule. The genes encoding these molecules are usually closely linked and transcribed as one precursor. In addition, there are often several copies dispersed throughout the genome, but the CN is generally less than 10 in prokaryotes (Figure 1; Liao 2000).

The functional eukaryotic ribosome is also composed of a small and a large subunit. The small subunit contains a *16-18S* rRNA molecule; the large subunit contains a *5S*, *5.8S*, and a *25-28S* rRNA molecule (Figure 2). The genes encoding *5.8S*, *18S*, and *28S* rRNA are generally found together in one or more tandem arrays of a repeat unit (rDNA) that consists of the three coding regions separated by intergenic spacers (IGS). These genes are transcribed as a single large precursor that is spliced to create the mature rRNA molecules that make up the core of the ribosomal subunits (Sollner-Webb & Tower 1986).

The CN of rDNA repeats can vary from as few as one copy per haploid genome in *Tetrahymena* to hundreds or thousands of copies, and it is positively correlated with genome size (Prokopowich et al. 2000). It is thought that the multiplicity of rRNA genes is required to offset the cell's inability to amplify the original transcript via multiple rounds of translation, as occurs with mRNA. Even so, most organisms

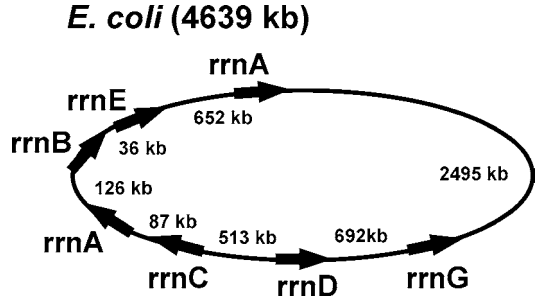

Figure 1 Diagram showing the orientation, distribution, and size, in nucleotides (expressed as kilobases—kb), of the seven rRNA operons in *E. coli* (modified from Liao 2000).

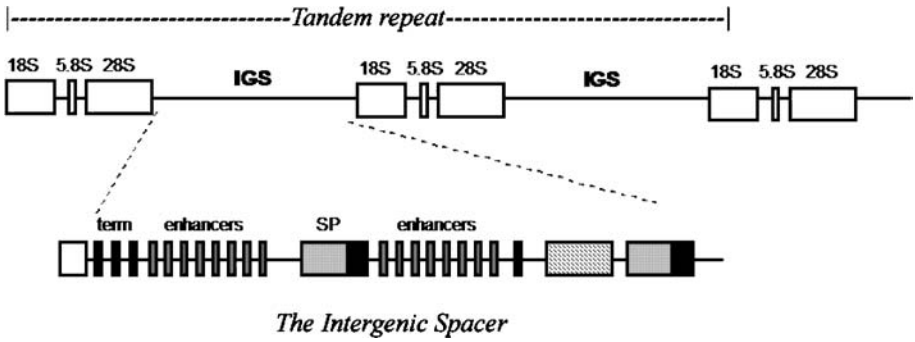

Figure 2 Diagram showing the basic structure of the genes encoding for eukaryotic rRNA (the rDNA). A single tandem repeat (plus a portion of an adjoining repeat) is shown. The intergenic spacer (IGS) region is highlighted to show the location of variable numbers of subrepeats (which often contain enhancers or promoters) that greatly influence IGS length heterogeneity (modified from Sterner & Elser 2002).

possess many more copies of rDNA than are needed to meet their requirement for rRNA production because some copies remain transcriptionally inactive even at maximal growth rate (Reeder 1999).

A high degree of sequence homogeneity is observed among rDNA copies within individuals and throughout entire species despite the divergence of these sequences between species. This pattern of sequence homogeneity, which was first described in the rDNA of *Xenopus* (Brown et al. 1972), is known as concerted evolution and is thought to be the result of molecular mechanisms such as unequal crossing over, gene conversion, and gene amplification, which have collectively been termed molecular drive (Dover 1982). Even so, variation among rDNA copies within species and within individuals has been observed, with respect to both nucleotide substitutions and length heterogeneity, the latter of which is often the result of variable numbers of subrepeats in the IGS. The rDNA repeat unit is composed of regions that are under different levels of selective constraint, and thus they vary substantially in their rates of divergence among species and in their levels of intraspecific polymorphism. For example, highly conserved core regions of the rRNA genes are interspersed with variable domains or expansion segments that show high levels of divergence between species, as well as variation within populations and individuals. Similarly, the IGS tends to show much higher levels of variation than do the core regions of the rRNA genes (e.g., Pikaard 2002).

Eukaryotic rDNA is one of the best-studied multi-gene families, and the results of this work have provided important insights into the mechanisms of concerted evolution (reviewed in Elder & Turner 1995, Liao 1999). For example, studies on *Drosophila* (Polanco et al. 1998, Schlötterer & Tautz 1994), *Daphnia* (Crease & Lynch 1991), humans (Seperak et al. 1988), and plants (Copenhaver & Pikaard 1996) have provided evidence that intrachromosomal recombination is much more

frequent than interchromosomal exchanges so that rDNA arrays within chromosomes tend to become homogenized into "superalleles," which then segregate within populations via sexual reproduction. In the case of some plant species with rDNA arrays on more than one chromosome, rates of exchange between the paralogous rDNA loci are so low that each array becomes homogenized for different variants (Rogers & Bendich 1987). Even so, the rDNA loci on these nonhomologous chromosomes are still more similar to one another than they are to orthologous rDNA loci in closely related species. This suggests that exchange does occur, albeit infrequently, between rDNA copies located on different chromosomes (Rogers & Bendich 1987).

Studies of rDNA variation in parthenogenetic organisms have shown that both intra- and interchromosomal exchanges occur in rDNA in the absence of meiosis and provide important opportunities for evolution within asexual lineages (Crease & Lynch 1991, Gorokhova et al. 2002, Hillis et al. 1991, Shufran et al. 2003). In addition, whereas many studies have suggested that unequal crossing over plays an important role in the generation of length variation among rDNA copies, as well as changes in overall CN of rDNA units, it is thought that this process alone cannot account for the rate of homogenization within rDNA arrays (Elder & Turner 1995; Liao 1999, 2000) and that its tendency to reduce the size of multi-gene families over time must be offset by other gene amplification mechanisms that are not yet well understood (Liao 1999, 2000; Elder & Turner 1995).

It is becoming increasingly clear that gene conversion plays a major role in the homogenization of multi-gene families and that natural selection may even favor the occurrence of initiation sites for gene conversion within the coding sequences of multi-gene families to ensure their homogenization (Liao 1999). Indeed, rates of gene conversion are estimated to be orders of magnitude higher than rates of unequal crossing over (Elder & Turner 1995, Liao 1999 and references within). In addition, Hillis et al. (1991) have shown that gene conversion in hybrid lizards can be biased and thus rapidly lead to the replacement of one variant or group of variants by another. It has also been suggested that most recombination events between members of multi-gene families on nonhomologous chromosomes occur via gene conversion (Liao 1999 and references within), an advantage of which is that such exchanges do not result in potentially deleterious gene rearrangements.

A number of other phenomena, which are beyond the scope of this review, but warrant mentioning, show how variation in rDNA structure and function can have far-reaching ecological and evolutionary consequences. For example, the presence of retrotransposable elements in rDNA (Eickbush 2002), which can lead to phenotypic abnormalities such as *bobbed* (Taylor 1923) or *abnormal abdomen* (Templeton & Rankin 1978) in *Drosophila*, can have major impacts on the fitness and survivorship of organisms (Hollocher & Templeton 1994, Templeton et al. 1993) and can be directly related to known environmental variables (e.g., temperature, rainfall) (Johnston & Templeton 1982). Likewise, work on the epigenetic phenomenon of nucleolar dominance in hybrids (Reeder 1985), whereby the rRNA genes of one parental species become transcriptionally dominant over the rRNA

genes of the other parental species, has been observed in a wide variety of organisms including amphibians (i.e., *Xenopus*), insects (i.e., *Drosophila*), numerous plant species, and even among hybrid mammalian somatic cell lineages (Reeder 1985). The functional ecological bases of phenomena such as nucleolar dominance remain to be studied in detail (Pikaard 2000).

FUNCTIONAL ECOLOGY OF rDNA VARIATION

Growth Rates and Rates of Transcription

PROKARYOTES Much of what is known about the role of rDNA in regulating rates of transcription and growth rates stems from studies done on prokaryotes, in particular, *E. coli* (Condon et al. 1995, Gourse et al. 1996, Sarmientos & Cashel 1983, Stevenson & Schmidt 1998), with much work about rate of ribosome synthesis and cellular growth rate being conducted in the 1960s (Maaløe 1969, Maaløe & Kjeldgaard 1966, Stent & Brenner 1961). The early work by Maaløe and others indicated that ribosome efficiency was relatively constant, leading many to conclude that growth rates were determined by the number of ribosomes in the cell. However, none of these experiments was performed under slow-growth conditions (Nomura 1999). Further work by Koch and colleagues (Koch 1971, Koch & Deppe 1971) demonstrated that ribosomes are in excess at low-growth rates. They argued that having unengaged ribosomes was advantageous for "shifting up" when and if substrates subsequently became available.

The rate-limiting step in ribosome synthesis at high-growth rates is the synthesis of rRNA. Gourse et al. (1996 and references within) argued that in order to make the very high number of ribosomes at high-growth rate, the promoters for the seven *E. coli* rRNA operons (Figure 1) transcribe greater than 50% of the cell's total RNA. This is remarkable given that there are approximately 2000 other operons in the cell. The central role of ribosome biogenesis in cellular function has motivated the development of a number of models to describe and explain the mechanisms responsible for regulating rRNA synthesis (Gourse et al. 1996). These models arbitrarily divide rRNA synthesis into different operating stages: (*a*) during steady-state growth at different growth rates (growth-rate-dependent control) and (*b*) during nutritional upshifts/downshifts and starvation conditions for amino acids (stringent control). Under growth-rate control of ribosome biosynthesis, free, nontranslating ribosomes inhibit the transcription of rRNA and tRNA genes (Maaløe 1969, Nomura 1999). However, under stringent control, uncharged tRNAs bound to RelA (relaxed control protein that is bound to ribosomes) cause it to produce guanosine-tetraphosphate (ppGpp), which inhibits rRNA and tRNA transcription (Nomura 1999, Wagner 1994).

EUKARYOTES The study of rRNA transcription has been ongoing for several decades, and, in fact, rRNA genes were one of the first sets of genes to be characterized and are now among the best-studied of all eukaryotic genes (Sollner-Webb &

Tower 1986). The typical cell in a eukaryote produces approximately 10,000 different RNA species, most of which are tRNAs and mRNAs. However, nearly 50% of a cell's transcriptional capacity (closer to 80% in yeast) (Moss & Stefanovsky 2002) is directed toward the synthesis of the ~*35-47S* rRNA precursor of the mature ~*18S*, ~*28S*, and *5.8S* RNAs of the ribosome. Even so, it is estimated that at maximum rRNA output, only about 50% of the rRNA genes are transcribed (Moss & Stefanovsky 2002). Indeed, it has been shown that yeast cells tend to alter rates of transcription of active genes rather than activate additional genes in response to increased requirements for rRNA (Reeder 1999 and references within). Even though rRNA transcription is known to be directly related to an organism's growth, development, and survivorship, it appears that the full transcriptional capacity of these genes is rarely (if ever) simultaneously engaged. The transcription of rDNA is catalyzed by RNA polymerase I (Sollner-Webb & Tower 1986), which is an efficient and highly controlled process. Each cell produces greater than a million new ribosomes each generation, which are required to support protein synthesis in the cells (e.g., for certain mammalian cells $\sim 2 \times 10^6$ ribosomes per ~15 h generation time) (Sollner-Webb & Tower 1986 and references within). The life span of an average ribosome is on the order of days to weeks (Moss & Stefanovsky 2002).

Variation in rDNA CN and IGS Length Heterogeneity

PROKARYOTES There can be a tremendous degree of variability in rDNA CN in nature and in laboratory cultures, and a number of studies have addressed the issue of whether variation in the numbers of rDNA genes (i.e., *rrn* operons) has adaptive significance in microbes. In laboratory experiments that varied temperature and shifted nutrient environments from simple to complex (i.e., varying carbon sources), Condon et al. (1995) found that increased rDNA copies increase the growth rate of *E. coli*, but only five of the seven *rrn* operons in *E. coli* (Figure 1) may actually be necessary for near-optimal, steady-state growth. However, redundant rDNAs were particularly beneficial when there was a rapid, favorable change in growth conditions (i.e., increased nutrients and/or temperature). The authors speculated that having multiple operons facilitates the surge in rRNA production induced by these new environmental conditions. Thus the significance of having seven *rrn* operons was not necessarily in supporting rapid growth rates but rather in allowing a rapid shift-up from one growth environment to a new environment.

Another important issue is whether there are any trade-offs associated with having too many copies of the rDNA operons. Similar to the findings of Condon et al. (1995), Stevenson & Schmidt (1998, 2004) found that an enhanced ability for a rapid response to favorable growth conditions occurred only when growth rates were moderate to fast. Under slow-growth-rate conditions, multiple operons appear to be nonadaptive. They noted that the presence of extra rDNA operons in the *E. coli* strain studied resulted in the overproduction of rRNA and decreased growth rates, especially under low-nutrient supply, suggesting that the regulation of rRNA synthesis was overwhelmed at slow-growth rates. This apparent disadvantage when

nutrient availability is low, i.e., slow-growth-rate conditions, may be compensated by the capacity to rapidly increase growth rate in a response to an influx of nutrients. The advantage of a rapid shift in growth rate may be rarely realized in stable, low-nutrient environments. However, it may occur under more unpredictable or fluctuating conditions, which may often be the case in natural bacterial assemblages (Koch 1971).

Multiple rDNA copies are common in other prokaryotes as well. In a survey of 76 bacterial genomes, Acinas et al. (2004) found that the maximum number of rDNA operons was 15 and the minimum, and most common, was 1. Among *E. coli* strains, CN can vary widely from 1 to 7 operons as cited above. Likewise, numbers can vary widely among *Vibrio* spp. and strains (Aiyar et al. 2002, Bag et al. 1999, Heidelberg et al. 2000). Aiyar et al. (2002) showed that *Vibrio natriegens,* with doubling times of less than 10 min, manages such rapid growth through a combination of mechanisms and that one of those mechanisms is to have high rDNA CN (up to 13). This study and several others have concluded that increased rDNA CN increases the speed at which bacteria can adapt to changing environmental conditions, but a high CN does not necessarily imply a high maximal growth rate at steady state (Klappenbach et al. 2000, Stevenson & Schmidt 2004). The results of these studies suggest that there are ecological trade-offs associated with rDNA CN, with more variable and nutrient-rich environments selecting for high-copy number and less variable, nutrient-poor systems selecting for lower CNs.

Another factor related to the ecological significance of rDNA is the efficiency with which the ribosomes function and, not surprisingly, different strains seem to function at different efficiencies. In a study of several prokaryotes, Cox (2004) demonstrated that increased growth of *E. coli* and *Streptomyces* was achieved via multiple copies of *rrn* operons, but that *Streptomyces* required higher ribosome content for similar growth rates, suggesting lower per-ribosome efficiencies. Such variations have some important ecological implications. For instance, nucleic acids are phosphorus (P)-rich (9–10% by mass) compared with the remainder of major cellular biochemicals (0–3% by weight) (Sterner & Elser 2002). Thus because most of the P in growing cells is associated with nucleic acids and, particularly, ribosomes, variability in ribosome efficiency should have important ecological feedbacks by altering the stoichiometric nutrient requirements of different microbial taxa (Elser et al. 2003, Makino & Cotner 2004, Makino et al. 2003) (see below). Of particular relevance was the observation by Elser et al. (2003) that a consortium of bacteria isolated from a Minnesota lake could grow at temperature-corrected rates similar to that of *E. coli*, but they achieved those growth rates using 20–50% less RNA. Therefore, one adaptation of growth in low-nutrient environments such as lake or sea water might be to increase ribosomal efficiency.

Although counterintuitive, there may be tighter regulation of rDNA transcription when there are multiple copies in the genome (Gu et al. 2003, 2004). For instance, some rDNA operons may function more constitutively, whereas others may be induced. In *Bacillus cereus,* which has 6–10 copies of rDNA, low-temperature-tolerant strains had unique rDNA sequence signatures relative to mesophilic strains,

suggesting different roles for some of the rDNA operons at low temperatures (Pruss et al. 1999). This idea was supported by a study demonstrating differential effects of particular rDNA deletions on *E. coli* growth rate and ribosome efficiency (Asai et al. 1999). Earlier work by Sarmientos & Cashel (1983) has shown that dual (tandem) promoters (*P1*, upstream; *P2*, downstream) on the *rrnA* operon of *E. coli* (Figure 1) are differentially regulated under fast- and slow-growth-rate conditions, suggesting the potential for adaptive responses in *E. coli*, and possibly in other prokaryotes.

EUKARYOTES Among eukaryotes, variation in CN has been studied in a variety of organisms (see below). In addition, many studies have focused on the role that length heterogeneity owing to variation in the number of subrepeats in the IGS, which often contain transcription promoter and enhancer sequences, plays in influencing rates of transcription and growth rate in a range of organisms (Flavell 1986, Reeder 1984).

Substantial pioneering work on rRNA transcription has involved the frog species, *Xenopus laevis* (Reeder 1984, 1985; Reeder & Roan 1984; Reeder et al. 1983). Reeder et al. (1983) injected *X. laevis* oocytes with ribosomal gene plasmids containing variable numbers of a particular (60/81 bp) IGS subrepeat element and found that (*a*) if a long IGS and a short IGS are allowed to compete in equal molar ratios, the gene promoter attached to the long IGS always has a higher rate of transcription (i.e., competition effect), and (*b*) as the total number of 60/81 bp subrepeats in the spacer of a given plasmid increases in a reaction, there is a concomitant decrease in the total amount of transcription (i.e., sink effect). Furthermore, the authors found that regardless of orientation, the 60/81 bp repeats still conferred competitive dominance on the longer IGS types. They proposed a model to suggest that the 60/81 bp repeats serve as "attraction sites" for some (as yet) unknown factors needed to stimulate/activate the gene promoter. This suggests that multiple mechanisms related not only to absolute IGS length but also to absolute number of particular repetitive elements (i.e., 60/81 bp elements) can influence the level/amount of transcription. Thus selection might favor some optimal or intermediate IGS length and rDNA CN, or a combination of the two.

ARTIFICIAL AND NATURAL SELECTION ON rDNA VARIATION

The preceding section has highlighted some aspects related to the functional significance of rDNA variations. We now examine changes in two main features of the rDNA in response to either artificial or natural selection: (*a*) rDNA CN and (*b*) rDNA IGS length that results from variable numbers of subrepeats. For the former, we simply refer to CN, whereas for the latter, we use the term length variant (LV). Table 1 summarizes a number of studies, including some examples that we discuss below.

TABLE 1 Compilation of studies that have observed changes in rDNA structure in response to either artificial selection or putative natural selection

Organism	Type of study	Observation	References
Mixed soil bacteria	Manipulated nutritional complexity of soil medium	Bacteria with higher average rDNA operon CNs grew more rapidly	Klappenbach et al. 2000
Fungi *Neotyphodium lolii* × *Epichlöe typhina* hybrid	Monitored strains in the lab for two generations	After only two generations of single-spore purification, there were significant shifts in IGS LVs suggesting that selection may favor longer LVs	Ganley & Scott 1998
Maize *Zea mays* L.	Artificial directional selection on increased yield	Shift in predominant IGS LVs to longer variants in certain strains	Rocheford et al. 1990 Kaufman et al. 1996
Barley—wild *Hordeum spontaneum*	Monitored strain yields in field populations for 54 generations	Significant shift in frequency of IGS LVs observed; may also be linked to other loci	Saghai-Maroof et al. 1984
Barley—wild *Hordeum spontaneum*	Surveyed distribution of genetic variants across habitats	Significant associations/correlations between IGS LVs and 8 of 9 physical factors in the environment	Saghai-Maroof et al. 1990
Barley wild—*Hordeum spontaneum* cultivated—*H. vulgare*	Surveyed distribution of genetic variants across habitats	Two rDNA locus (*rrn-1* and *rrn-2*) genotypes that differed in IGS LVs were correlated with 9 physical factors in the environment	Allard et al. 1990 Zhang et al. 1990
Barley wild—*Hordeum spontaneum* cultivated—*H. vulgare*	Surveyed natural variation in rDNA LVs.	Significant associations between IGS LVs and microsite features (humidity, rainfall, edaphic factors) suggest that selection is operating along a humidity/aridity gradient	Gupta et al. 2002 Sharma et al. 2004

Barley—wild *Hordeum spontaneum* and F_1 hybrids	Artificial selection on yield and associated other reproductive traits	Yield and other traits were strongly associated with alleles at the *NOR-H3* locus, indicating that a small section of genome associated with nucleolar organizing regions has a strong effect on reproductive traits	Powell et al. 1992
Oats—wild *Avena barbata*	Natural survey of genetic variation at rDNA loci and multi-locus allozymes	Seven different habitat-genotypes/ecotypes were detected including rDNA/allozyme/morph correlations along a moisture/rainfall gradient. Ecological diversification occurred over a relatively short (150–200 generations) time span	Cluster & Allard 1995
Oats—wild *A. barbata* and other crop species	Survey of literature focusing on *A. barbata* rDNA IGS variation	Non-random distribution of IGS LVs among different species suggests that an optimal (intermediate) IGS LV may exist for many species	Jorgensen & Cluster 1988
Oats—wild *A. barbata*	Glasshouse competition experiments and quantitative genetic analyses	Follow up on earlier studies of allozyme/rDNA associations found that mesic genotype outcompeted xeric genotype: crossing experiments showed significant heritabilities for life history traits along a moisture gradient	Latta et al. 2004
Oats—cultivated *Avena sativa*	Breeding studies of cultivars	Cultivars/strains bred in controlled studies (for higher yield) had significantly longer IGS LVs than wild strains	Polanco & Pérez de la Vega 1997
Emmer wheat—wild *Triticum dicoccoides*	Natural survey of rDNA IGS LVs	Suggestive evidence that selection is operating in the IGS with weaker support for selection on total rDNA copy number	Flavell et al. 1986

(*Continued*)

TABLE 1 (*Continued*)

Organism	Type of study	Observation	References
Rice—wild and cultivated *Oryza* spp.	Monitored genetic variation in cultivated and wild populations	Significant differences in IGS LVs between cultivated and wild rice species. Cultivated African rice (*Oryza glaberrima*) showed no variation in IGS LVs compared with two wild species; suggestive of purging/purifying in IGS LVs among cultivated species?	Cordesse et al. 1990
Pitch pine *Pinus rigida* Mill.	Natural genetic variation in rDNA CN was assayed	A strong inverse correlation between rDNA CN and level of environmental stress (fire-related) suggests that high stress may be correlated with low CN	Govindaraju & Cullis 1992
Red spruce—*Picea rubens* Black spruce—*P. mariana*	Surveyed natural population genetic variation in rDNA repeat unit types and rDNA CN	Distinct rDNA alleles (repeat types) occurred across a latitudinal gradient with threefold and sixfold variation in copy number for *P. rubens* and *P. mariana*, respectively. Suggests possible role for selection (i.e., temperature, growing season length)	Bobola et al. 1992
Douglas fir *Pseudotsuga menziesii*	Natural variation in rDNA copy number across latitudinal, longitudinal and elevational gradients	Positive (weak) association between rDNA copy number and latitude (higher copy number in more northern populations) and longitudinal trend with coastal (western) populations having higher CNs	Strauss & Tsai 1988
Fruit fly *Drosophila melanogaster*	Disruptive artificial selection for increased or decreased preadult development rate	Long IGS LVs predominated in fast-developing lines, while the opposite occurred in slow-developing lines	Cluster et al. 1987

Fruit fly *D. melanogaster*	Monitored genetic structure of natural populations along a latitudinal range	Significant clinal shifts in distribution of IGS LVs among females across the latitudinal gradient suggests that selection exerts pressure on rDNA structure (perhaps temperature-related)	Polanco et al. 1998
Aphid/greenbug *Schizaphis graminum*	Artificial selection for insecticide (disulfoton) resistance for 200 generations (~4 years)	Control lines showed no shifts; selected lines showed shifts in IGS LVs with the loss of three LVs from the original population. Indicates the potential for large-scale genetic changes in a parthenogen	Shufran et al. 2003
Grasshopper *Dichroplus elongates*	Monitored natural genetic variation across an altitudinal gradient	The average number of RFLP rDNA variants (primarily in the IGS) per individual was significantly associated with altitude with individuals from higher altitudes expressing greater numbers of rDNA variants	Clemente et al. 2002
Water flea *Daphnia pulex*	Artificial disruptive selection on growth-related life-history features	Significant changes in predominant IGS LVs with higher proportions of longer LVs in lines exhibiting higher juvenile growth rate. Concomitant changes in percentage of RNA and phosphorus were also observed	Gorokhova et al. 2002
Frog *Xenopus laevis*	Laboratory in vitro experiments	Longer IGS LVs were more competitive than shorter LVs in a series of in vitro transcription assays	Reeder et al. 1983
Chicken several specialized strains *Gallus gallus*	Artificial selection experiments on yield and growth	Shifts in IGS regions and nucleolar (NOR) loci were related to selection for either somatic or reproductive growth	Delany & Krupkin 1999, Su & Delany 1998

Artificial Selection

The relationship between rDNA structure and important fitness-related traits (e.g., growth rate, productivity/yield) in both wild and domesticated populations has been studied extensively in crop science, where artificial selection on specific life-history traits has been conducted for a variety of species including maize (Kaufman et al. 1996, Rocheford et al. 1990), barley (Powell et al. 1992, Saghai-Maroof et al. 1984), oats (Latta et al. 2004, Polanco & Pérez de la Vega 1997), and rice (Cordesse et al. 1990). In addition, artificial selection experiments have been conducted on a few model animal species, including invertebrates such as the fruit fly *Drosophila melanogaster* (Cluster et al. 1987), the aphid/greenbug *Schizaphis graminum* (Shufran et al. 2003), and the water flea *Daphnia pulex* (Gorokhova et al. 2002), as well as vertebrate models such as the frog *X. laevis* (Reeder et al. 1983) and the chicken (Delany & Krupkin 1999, Su & Delany 1998). In general, these studies have documented significant changes in rDNA structure in response to selection on important life-history/fitness-related traits (e.g., Kaufman et al. 1996, Powell et al. 1992, Rocheford et al. 1990, Saghai-Maroof et al. 1984) (see Table 1). Furthermore, quantitative genetic studies among certain crop species (e.g., barley, wild oats) have attempted to pinpoint key ecological traits among rDNA genotypes in wild populations to elucidate the quantitative genetic underpinnings of this variation (Latta et al. 2004, Powell et al. 1992).

Among the few animal studies that have utilized artificial selection on specific traits (e.g., growth rate, life-history traits) and examined concomitant changes in rDNA, the studies by Cluster et al. (1987) and Grimaldi & Di Nocera (1988) on *D. melanogaster* are noteworthy because they show a clear response of the rDNA to directional selection. Cluster et al. (1987) selected for fast and slow development times among lines of *D. melanogaster* and noted a significant shift in the frequency of IGS LVs in the two selection regimes. A greater proportion of the faster developing lines maintained longer LVs, whereas the slower developing lines maintained shorter LVs. Subsequent work by Grimaldi & Di Nocera (1988) showed that the rate of transcriptional production of pre-rRNA was directly proportional to the number of enhancers located in the IGS. These two studies provide support for the notion that genotypes composed of longer IGS LVs may benefit from higher rDNA transcriptional rates via more enhancer and promoter sites in the subrepeat region of the IGS and thus exhibit faster development (higher growth rates).

In another study using the cyclically parthenogenetic water flea *Daphnia pulex*, Gorokhova et al. (2002) observed shifts in the frequency of IGS LVs during direct selection on a life-history character (production rate) in progeny descended from a single (clonal) stem mother. The frequency of a longer LV increased as production/fecundity rate decreased, along with concomitant shifts in other important characters such as the percentage of RNA, and body phosphorus and juvenile growth rates (see below). These data clearly show that even within a single clone of a parthenogenetic species considerable plasticity exists in the ability to shift key life-history features along with LV frequencies in response to artificial selection.

This implies that asexual organisms possess considerable flexibility to adapt to changing environmental conditions via structural mutations in the rDNA.

Finally, work with domesticated strains of chicken (Delany & Krupkin 1999, Su & Delany 1998) has shown that in some artificial selection experiments, the combination of shifts in both IGS length variation and overall rDNA CN need to be considered jointly before any predictions can be made about the outcome of the selection regime.

Natural Selection

A number of studies (Table 1) have surveyed and/or examined natural variation in rDNA CN and/or LVs in a number of organisms, including soil bacteria (Klappenbach et al. 2000), wild barley (Allard et al. 1990, Gupta et al. 2002, Saghai-Maroof et al. 1990, Sharma et al. 2004, Zhang et al. 1990), wild oats (Cluster & Allard 1995, Jorgensen & Cluster 1988), wild emmer wheat (Flavell et al. 1986), a variety of conifer species (Bobola et al. 1992, Govindaraju & Cullis 1992, Strauss & Tsai 1988), and arthropods (Clemente et al. 2002, Polanco et al. 1998), and have attempted to relate this underlying genetic variation to potentially important environmental variables.

Among prokaryotes, Klappenbach et al. (2000) found that in a phylogenetically diverse soil bacterial assemblage, species that possessed higher average CNs of rDNA genes formed colonies more quickly when exposed to nutritionally complex medium, as opposed to species that possessed lower average CNs, which responded more slowly. They concluded that rDNA CN strongly influences ecological strategies and competitive abilities among soil bacteria and potentially can be under direct natural selection.

Among eukaryotes, work on cultivated and wild barley, *Hordeum vulgare* and *Hordeum spontaneum*, respectively, has shown strong correlations between LV composition and important environmental/selective factors such as temperature and moisture availability/humidity (Allard et al. 1990, Gupta et al. 2002, Saghai-Maroof et al. 1990, Zhang et al. 1990). Allard et al. (1990) suggested that selection is acting directly on the sequence variability in the transcription units (i.e., subrepeats in the IGS), but no eco-physiological traits were assessed. In a companion paper, Zhang et al. (1990) determined that the high adaptedness associated with a few specific alleles may result from adaptively favorable nucleotide sequences in either the transcription units or the IGS and that adaptedness in barley depends more on the quality (i.e., sequence and length variation) rather than the quantity (i.e., CN) of rDNA present. Similar work on another important crop species, wild emmer wheat (*Triticum dicoccoides*), reported by Flavell et al. (1986), provides further support for the notion that natural variations at rDNA loci are significantly correlated with important environmental parameters. Govindaraju & Cullis (1992) examined rDNA CN and LVs in eight populations of pitch pine (*Pinus rigida* Mill.) associated with the Pine Barrens region of southern New Jersey. Interestingly, the authors noted an inverse relationship between rDNA CN and the level of

environmental stress and proposed that strong diversifying selection is operating to influence rDNA CN in this species. Although this study is purely correlational, it suggests that rDNA CN may indeed be influenced by environmental parameters, akin to the soil microbe study of Klappenbach et al. (2000) cited above.

From the above examples and others listed in Table 1, it indeed appears that variations in rDNA CN and LVs in a broad range of organisms have ecological significance and can respond to either artificial or natural selection.

LINKING rDNA VARIATION TO ECOLOGICAL PROCESSES

A Stoichiometric Perspective

The previous sections have highlighted how structural and regulatory features of rDNA operate to affect key aspects of organism function, such as the maximal and realized rates of growth and development of diverse biota ranging from bacteria to vertebrates. In particular, we have seen that variations in rDNA structure and expression are commonly linked to the challenge of maintaining a high rate of rRNA production associated with rapid cellular proliferation. In this section we show how the functional consequences of rDNA variation extend well beyond those normally considered by cellular and evolutionary biologists. We employ the perspective of biological stoichiometry (the study of the balance of energy and multiple chemical elements in living systems) (Sterner & Elser 2002) to consider how ecological forces, such as the supply of the key limiting nutrient phosphorus (P), impinge on evolutionary change involving rDNA, as well as potential feedbacks generated by the coupling of rDNA to growth and ribosome production. In doing so we highlight some of the ecological forces that may operate to impose trade-offs on the evolution of high-growth-rate phenotypes associated with changes in the rDNA genome. Connections between growth, cellular P requirements, and RNA allocation under conditions of environmental P limitation have been established for some time in unicellular algae, dating to classic studies of Rhee and colleagues (e.g., Rhee & Gotham 1981 and references within). These connections have more recently become integrated in various emerging stoichiometric models of growth-rate regulation in photoautotrophic organisms (e.g., Ågren 2004, Klausmeier et al. 2004).

Because the associations of growth, RNA, and P in autotrophs have been covered extensively elsewhere (Frost et al. 2005, Geider & La Roche 2002, Sterner & Elser 2002), our emphasis in this section is on connections between these variables in metazoans, where the elemental composition of animal biomass, its physiological regulation, and its connections to biochemical allocations have come under close scrutiny in only the past 10 years. It is now known that the elemental composition of animal biomass, in contrast to the physiological plasticity of autotrophs, is homeostatically regulated by various physiological mechanisms around taxon- and stage-specific levels (Sterner & Elser 2002). For example, the P content of the ubiquitous crustacean zooplankter, *Daphnia*, varies between only 1.2 and 2%, with variation largely due to the stage of development (juveniles have somewhat higher

P content than adults). In contrast, another crustacean zooplankter, *Bosmina*, generally seems to have lower P content, with values between 0.6 and 0.9% (Hessen & Lyche 1991). Such studies have been extended recently to include insects and show that crustacean zooplankton and insect taxa exhibit similar ranges of variation in C:N, C:P, and N:P ratios (Elser et al. 2000b). In the following we focus on microorganisms (heterotrophic bacteria) and invertebrate animals (primarily insects and crustaceans). We choose this emphasis because in vertebrate animals the dominant form of P is in the apatite mineral that forms bone (Elser et al. 1996) and thus potential connections to rDNA variation are obscured.

Because variation in C:N:P ratios has been shown to have considerable consequences for ecological processes such as secondary production (Sterner & Schulz 1998) and consumer-driven nutrient recycling (Elser & Urabe 1999), a desire to understand the biological basis of this variation led to formulation of the growth-rate hypothesis (GRH) (Elser et al. 1996), which states that variation in organismal C:N:P ratios reflects differences in growth rate because of differential allocation to P-rich rRNA (RNA is ~9.6% P by mass) that is needed to meet the protein synthesis demands of growth. Elser et al. (2000b) extended the GRH to include its genetic basis, postulating that variations in rDNA IGS length and CN underpin variation in growth and therefore RNA allocation, and thus P content and C:N:P ratios.

So, is growth-related variation in RNA allocation sufficient to explain organism level variation in P content? A variety of recent studies indicates that this is the case, at least in microorganisms (Makino et al. 2003) and invertebrates including zooplankton (Acharya et al. 2004; Carrillo et al. 2001; Elser et al. 1996; Main et al. 1997; Vrede et al. 1998, 2002;) and insects (Schade et al. 2003). In an integrated analysis, Elser et al. (2003) showed not only that growth, RNA, and P were generally tightly coupled across all study organisms (ranging from microbes to invertebrates), but also that RNA allocations were sufficiently large such that RNA contributed on average ~50% of biomass P across the taxa reported. Thus RNA production generates a physiologically dominant pool of P in many organisms and therefore rDNA transcription itself appears to represent an ecologically and biogeochemically significant process.

This work supports the hypothesized connection among organismal growth rate, RNA allocation, and C:N:P stoichiometry proposed nearly 10 years ago (Elser et al. 1996). Thus uncovering the contributions of rDNA variations that drive variation in growth and RNA allocation will be fruitful in many areas. This empirical base has now stimulated several lines of theoretical investigation of the GRH (Ågren 2004, Klausmeier et al. 2004, Vrede et al. 2004). These papers offer different perspectives on this coupling, but their collective message is that relatively simple eco-evolutionary formulations are beginning to capture the ecological significance of RNA-related impacts on C:N:P stoichiometry and growth rate. For example, under resource-rich conditions, increased allocation to assembly machinery (i.e., RNA) results in organisms with low optimal N:P ratios (that is, organisms that are easily P limited and are poor P competitors) whereas low-resource environments favor organisms with high optimal N:P ratios (Klausmeier et al. 2004).

We now turn our attention to the question of whether connections, indeed, exist among rDNA variations and C:N:P stoichiometry. In particular, Elser et al. (2000b) proposed that there should be a generally positive association between the length of the rDNA IGS and organismal growth rate, RNA content, and P content in eukaryotes. The data collected for the explicit purpose of evaluating these connections remain quite limited; however, a number of relevant studies have appeared (discussed, in part, above). For example, Weider et al. (2004) identified IGS length variation among clones in three species of *Daphnia* and examined growth rates, RNA levels, and P contents under standardized conditions. As predicted by the GRH, there were positive correlations between RNA:DNA ratio and either growth rate or IGS length, and a significant positive correlation between IGS length and growth rate when clonal means for all three species were examined. However, no clear-cut relationships between RNA:DNA and P content were observed for any of the three species, likely because of the limited sample size and narrow range of values observed for P content and RNA:DNA ratios. However, as noted above (Gorokhova et al. 2002), IGS length in single daphnid clones can respond to artificial selection with concomitant changes in growth rate, RNA, and P content.

The aforementioned studies provide some of the first evidence that rDNA variations can be connected not only to growth itself but also to the elemental composition of living biomass. This suggests the existence of a potentially important mechanism for a trade-off in the evolution of rapid growth rate: high-growth rate appears to impose a disproportionate elevation in organismal P demands in order to maintain production of P-rich rRNA. Preliminary evidence from evolutionary/ecological studies provides some support for this claim. For example, there is a general trend for increasing growth and development rates for organisms as one moves to high latitudes (Conover & Schultz 1995), suggesting that arctic biota should be more P-rich than those in temperate and tropical regions, a pattern documented in recent studies of vascular plants (McGroddy et al. 2004, Reich & Oleksyn 2004) and in *Daphnia* (Elser et al. 2000a). Indeed, the results of feeding experiments in the latter study showed that arctic *Daphnia* were not only more P-rich than their temperate counterparts, they also were more sensitive to low P in their diets and had very low recycling rates of P. Weider et al. (2005) have recently evaluated whether stoichiometric food quality impinges on the relative success of rDNA variants. In an experiment where algal food differing in C:P ratio was externally supplied to a mixture of two allozymically different *D. pulex* clones differing in the length of their IGS, the clone with the longer IGS (and likely to have higher P requirements according to the GRH) increased dramatically relative to the clone with the shorter IGS when the external food supply was P-rich. Conversely, when food was low in P, the clone with the shorter IGS, which is likely to have lower P requirements according to the GRH, won out. In a follow-up experiment, algal food was not supplied externally, but instead algae and *Daphnia* were present together and thus nutrient recycling by the *Daphnia* could play a role. In this case, clonal coexistence of these two variants was observed in treatments of different light intensity that were intended to produce divergence in algal C:P ratios. Thus

feedback mechanisms at the ecosystem level may contribute to stabilizing the coexistence of rDNA variants in nature. The above studies highlight that connections between rDNA variation, C:N:P ratios, and important ecological parameters (e.g., growth rate, competitive ability) may indeed be important and certainly warrant further study.

CONCLUDING REMARKS

In this review, we have provided evidence from a variety of organisms that selection on variation in the CN or IGS length of rDNA can have a number of substantial ecological consequences. This is because rDNA polymorphisms can affect two aspects of profound ecological importance: (*a*) growth rate, which is directly linked to ecological production, and (*b*) organismal P requirements, which contribute to stoichiometric food quality effects and the sequestering and recycling of phosphorus. Therefore, patterns of energy flow and the cycling of a key limiting nutrient (P) are simultaneously impacted by evolutionary events mediated by the functional consequences of rDNA variation. Taking such a multilevel approach as indicated above may allow us to begin linking subcellular and genetic processes (as exemplified by rDNA variation, and its impacts on ribosome biogenesis) with the evolution of major life-history traits and, ultimately, lead to a better understanding of the nature and outcome of ecological interactions in natural ecosystems.

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