Do phosphorus requirements for RNA limit genome size in crustacean zooplankton?

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Abstract: As for most other organisms, genome size in zooplankton differs widely. This may have a range of consequences for growth rate, development, and life history strategies, yet the causes of this pronounced variability are not settled. Here we propose that small genome size may be an evolutionary consequence of phosphorus (P) allocation from DNA to RNA under P deficiency. To test this hypothesis we have compared the two major groups of zooplankton, copepods and clado-cerans, that have overlapping niches and body size. Relative to the cladocerans, copepods have a more complex life history and a lower mass-specific P content, while cladocerans tend to have higher P and RNA contents and higher specific growth rates and frequently experience P-limited growth, likely due to a shortage of P for ribosome synthesis. Cladocerans also generally have smaller genomes than copepods (1C = 0.17–0.63 pg DNA·cell^{-1} vs. 1C = 0.10–10 pg DNA·cell^{-1}). Further-more, cladocerans have a higher slope of the relationship of body size with DNA content (1.5 vs. 0.28 in copepods) and present almost 15-fold higher RNA:DNA ratios (24.8 in cladocerans vs. 1.6 in copepods). Hence, small genome size in cla-docerans could reflect an evolutionary pressure towards "efficient" genomes to conserve a key element needed to maxi-mize growth rate. We do not claim that this is a universal cause of genome size variability, but propose that streamlining of genomes could be related to P conservation rather than energy conservation. This could be relevant for a range of organ-isms that may suffer P-limited growth rates.

Key words: cladoceran, copepod, genome size, phosphorus, RNA.

Résumé : Comme chez la plupart des autres organismes, la taille du génome varie considérablement chez le zooplancton. Ceci peut avoir toute une série de conséquences sur le taux de croissance, le développement et les stratégies de cycle vital. Pourtant, les causes de cette variabilité prononcée ne sont pas établies. Les auteurs proposent ici qu'un génome de petite taille serait une conséquence évolutive de l'allocation préférentielle du phosphore (P) dans l'ARN plutôt que l'ADN dans des conditions de déficience en P. Pour vérifier cette hypothèse, les auteurs ont comparé les deux grands groupes de zoo-plancton, les copépodes et les cladocères, qui présentent des niches et des tailles corporelles qui se chevauchent. Par rap-port aux cladocères, les copépodes ont un cycle vital plus complexe et un faible contenu en P en fonction de leur masse tandis que les cladocères tendent à afficher des teneurs plus élevées en P et en ARN, des taux spécifiques de croissance plus élevés et ils rencontrent plus fréquemment une limitation de leur croissance due à une pénurie de P, vraisemblable-ment en raison d'un manque de P pour la synthèse des ribosomes. Ils présentent aussi généralement des génomes plus pe-tits par rapport aux copépodes (1C = 0,17–0,63 pg d'ADN/cellule vs. 1C = 0,10–10 pg d'ADN/cellule). De plus, chez les cladocères, la pente de la relation entre la taille corporelle et le contenu en ADN est plus grande (1,5 vs. 0,28 chez les co-pépodes). Ainsi, la petite taille du génome chez les cladocères pourrait refléter une pression évolutive favorisant les géno-mes plus « efficients » afin de conserver un élément clé requis pour maximiser le taux de croissance. Les auteurs n'affirment pas qu'il s'agit là d'une cause universelle de la variabilité de la taille des génomes, mais proposent plutôt que la minimisation des génomes pourrait résulter d'une conservation du P plutôt que d'une conservation de l'énergie. Ceci pourrait s'avérer pertinent pour toute une gamme d'organismes qui peuvent connaître une limitation de leur taux de crois-sance découlant d'une déficience en P.

Mots-clés : cladocères, copépodes, taille du génome, phosphore, ARN.

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Introduction

Genome size in organisms varies tremendously, but there is no consensus on the causes of this variability (Cavalier-Smith 1978; Gregory et al. 2000). In principle, two major explanations have been advocated, the "bulk DNA" and the

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"selfish DNA" hypothesis (Lynch 2007). As with any organismal trait, part of this variability could reflect phylogenetic constraints due to past evolutionary adaptations. However, closely related taxa can have widely different genome sizes (Hardie and Hebert 2004; Rees et al. 2007), pointing towards recent evolutionary events and thus other mechanisms. Large body size seems to be associated with larger genomes and vice versa within some phyla (Gregory 2001), yet the direction of causality for this correlation is not straightforward (Rasch and Wyngaard 2006). There can also be considerable variability in genome size among organisms of similar body size (Gregory et al. 2000) or even at the intraspecific level owing to environmental conditions or ontogeny (McLaren et al. 1988; Nardon et al. 2003). Such differences in genome size may be attributed to evolutionary adaptations reflecting different life cycle strategies or differences in complexity of body structure or life cycles. Hence, despite a wealth of studies, there are no unifying explanations for the variability in genome size, which could reflect that there are a number of different mechanisms that affect genome size.

Much effort has been devoted to explaining the frequently abundant quantities of non-coding DNA in genomes, describing this non-coding DNA as "junk" or selfish DNA (Dawkins 1976; Orgel and Crick 1980). While it has recently become clear that some non-coding DNA regions may actually serve an important role in gene regulation, it is not clear to what extent these regions promote fitness at the organism level. An obvious correlate of increased genome size is increased cell size. In addition, larger cells seem to require larger nuclei for more efficient mRNA transport into the cytoplasm (Cavalier-Smith 1985). Large cell size generally reduces cellular metabolic rate and cell division rate (Gregory et al. 2000; Rasch and Wyngaard 2006). Hence, one would expect that small body size, rapid growth, and simple life cycles would promote small genome size. There might, however, also be other mechanisms linked with allocation of energy or matter that could affect genome size.

Some past considerations of the evolutionary basis of variation in genome size have examined the possible material or energy costs of constructing and maintaining the genome. In considering the widespread prevalence of haploidy in the diatoms, Lewis (1985) proposed that haploidy provides an advantage to diatoms in nutrient competition because it reduces (by 50%) the nutrients (N, P) that must be allocated to the genome, allowing them to be allocated elsewhere (such as to RNA) in support of growth. Of particular interest in considering the construction costs of DNA is the observation that nucleic acids are especially P-rich compared with other molecules in the cell (Sterner and Elser 2002). This suggests that variations in genome size might be of particular ecological importance, and thus subject to selection, in organisms that potentially experience frequent P limitation of their growth rate. On the basis of this reasoning, here we propose, and provide a preliminary test of, a novel hypothesis to explain some variation in genome size: *intracellular allocation of P from DNA to RNA results in decreased genome size and elevated RNA:DNA ratio*. This is a function of organism growth rate and thus its demands for maximizing RNA

and is likely to be strongly favored by P limitation. To test this hypothesis, one should compare relatively closely related taxa of similar body size that live in similar environments but which differ in growth rate and potential P limitation of growth rate.

Our test of this idea involves crustacean zooplankton. Crustaceans show a truly remarkable variability in genome size. Within the order Amphipoda, a 20-fold range ($1C = 2.94$–64.6 pg DNA·cell^{-1}) has been recorded (Rees et al. 2007). The large subclass Copepoda also shows a striking variability in genome size ($1C = 0.10$–12.46 pg DNA·cell^{-1}), with generally larger genomes in the order Calanoida compared with the order Cyclopoida, yet cyclopoids may also show a 20-fold range ($1C = 0.10$–2.02 pg DNA·cell^{-1}) (Rasch and Wyngaard 2006). The small genome size of certain cyclopoids has been attributed to evolutionary adaptations for rapid replication, accelerated development, and survival in unpredictable habitats (Rasch and Wyngaard 2006).

In contrast to the wide variability exhibited by copepods, members of the order Cladocera (class Branchiopoda), key players in lake ecosystems, are characterized by generally very small genome sizes ($1C = 0.17$–0.63 pg DNA·cell^{-1}), according to the Animal Genome Size Database (Gregory 2008, www.genomesize.com), with the majority of species having <0.4 pg DNA·cell^{-1}. Cladocera are characterized by simple life cycles (no larval stages, direct development, and facultative parthenogenesis), rapid growth rates, and high body P content (percentage of dry mass). While they are generally the most abundant group of crustaceans in freshwaters both numerically and in terms of biomass (Hessen et al. 2006), there are only a few marine species, primarily in brackish waters. In these fast-growing invertebrates, a major fraction (50%–80%) of cellular P is allocated to RNA owing to a high demand for ribosomes to drive protein synthesis (Elser et al. 2003; Hessen et al. 2007). For this reason, and because there often is a relative scarcity of P in plant biomass relative to the demands of invertebrate herbivores, grazers with high maximum growth rates may face P-limited growth. This has been experimentally verified for various invertebrates, notably in the key cladoceran family Daphniidae (cf. Sterner and Elser 2002). Copepods, on the other hand, have obligate sexual reproduction, go through a number of developmental stages, and may have multivoltine life cycles. Hence they generally have slower growth rates and lower specific P content than the Cladocera (Andersen and Hessen 1991). Extensive tests for P limitation of copepod growth have not yet been performed. However, in one study (Villar-Argaiz and Sterner 2002), low-P algal food had little impact on the growth and development of various naupliar and copepodid stages, except for the copepodite instar CII, which were unable to molt when raised on P-deficient food. Copepodite nauplii are rich in P, however, and their growth rates are closely linked with their specific P content (Carrillo et al. 2001). In sum, available data suggest that cladocerans and copepods differ considerably in their life history strategies and in their likely susceptibility to P limitation. This provides an opportunity to evaluate our ideas. Here we provide a preliminary test of our P-allocation hypothesis via compilation of a large data set of published values of specific DNA and RNA contents

for species of cladocerans and copepods to see whether there are differences in C-values that are consistent with different strategies for P allocation between DNA and RNA. The mechanisms outlined in our proposal for cladocerans and copepods are not specific to these groups. Thus, our findings could also be relevant for explaining the remarkably different genome sizes found in other taxa subject to the same selection pressure, i.e., dietary P deficiency and demands for high growth rates.

Material and methods

Published values of specific DNA and RNA contents (percentage of dry mass) for cladocerans and copepods were obtained from Ventura (2006). These include a wide range of species, and for copepods, both freshwater and marine calanoids and cyclopoids were included. C-values (the quantity of nuclear haploid DNA) used in this study are derived from published sources (Gregory 2008, www.genomesize.com; Rasch and Wyngaard 2006). C-values are based on various somatic tissues, generally from a mixture of cell types from ground-up animals. The percentages of body P contributed by DNA and RNA were estimated by multiplying their concentrations by their P fraction (0.089 and 0.085 for DNA and RNA, respectively; Ventura 2006) and the total nucleic acid P is the sum of P in both DNA and RNA.

To test for a potential confounding effect of body size on DNA content (estimated from C-values and the number of genome copies in each species) and RNA:DNA ratios, we obtained data on maximum body length for all species. Length was used as an estimator of body size in both groups because of its good allometric relationship with biomass (McCauley 1984). For Cladocera, this length was the distance between the upper edge of the head and the base of the tail spine; for copepods, length was from the anterior end of the cephalothorax to the posterior end of the furca. For copepods, we used the data provided by the authors describing C-values converted to maximum length when only prosome length was reported. In cladocerans, since body sizes were not available from studies reporting C-values, we used the maximum body size for each species described in various taxonomic sources (Brooks 1957; Negrea 1983; Hebert and Finston 1993, 1996, 1997; Alonso 1996; Flössner 2000).

Results

Published C-values show considerably lower variability in cladocerans than in copepods (Fig. 1a). In support of previous observations, there was a consistent difference in P content (percentage of dry mass) between cladocerans and copepods (one-way ANOVA, $F_{49,1} = 8.1$, $P = 0.006$), with averages ± SD of 1.26% ± 0.41% and 0.93% ± 0.42%, respectively (Fig. 1b). The contrasting allocation of P to RNA or DNA in cladocerans and copepods is reflected in the widely different RNA:DNA ratios for the two groups (Fig. 1c; 24.8 ± 16.6 and 1.6 ± 1.2 for cladocerans and copepods, respectively). The difference between these two groups of planktonic crustaceans becomes even more striking when the P is assigned to DNA and RNA (Fig. 1d). While RNA is the dominant pool of P in both groups (average ± SD: 0.45% ± 0.09% and 0.25% ± 0.13% of dry

mass for cladocerans and copepods, respectively), the relative fraction of body P allocated to RNA is significantly higher in cladocerans, while the fraction of P in DNA for cladocerans (0.03% ± 0.01%) is strikingly low and significantly lower than that in copepods (0.15% ± 0.09%) (Fig. 1d). However, on average, P in total nucleic acids represents 0.38% ± 0.08% of dry mass in copepods and slightly (but not significantly) more in the Cladocera (0.48% ± 0.2%).

To test for potential body size effects on the pattern observed in Fig. 1, we regressed genome size against maximum body size for all species where C-values were available (Table 1). For both groups we detected a strong positive correlation between body size and genome size (Figs. 2a, 2b), but the slopes were strikingly different (1.5 for cladocerans vs. 0.28 for copepods; Table 1) (ANCOVA, $F_{74,1} = 40.469$, $P < 0.0001$ and ANCOVA, $F_{74,1} = 9.659$, $P = 0.003$ for the intercept and slope, respectively) in spite of both groups covering the same size range. Thus, in comparison with copepods, large cladocerans have considerably smaller genomes than one would expect for their body size. We also note that calanoids and cyclopoids also had significantly different slopes for body size vs. DNA relationships (0.42 vs. 0.28, respectively; Table 1) (ANCOVA, $F_{39,1} = 5.884$, $P = 0.02$ and ANCOVA, $F_{39,1} = 21.806$, $P < 0.0001$ for the intercept and slope, respectively). The difference in DNA content (2C) between cyclopoids and calanoids seemed primarily to be due to smaller body size in cyclopoids (Fig. 2a). Among calanoids there were both freshwater and marine species, while all cyclopoids and cladocerans were from freshwater habitats. Freshwater and marine calanoids had nonsignificantly different slopes (ANCOVA, $F_{28,1} = 0.025$, $P = 0.875$ and ANCOVA, $F_{28,1} = 0.452$, $P = 0.452$ for the intercept and slope, respectively), which indicated that habitat was not a relevant factor for this comparison. Within the cladocerans, most of the species analyzed for genome size were from the genus *Daphnia*, although the data set covered genera from 3 of the 4 suborders of the Cladocera (*Bosmina*, *Ceriodaphnia*, *Daphniopsis*, *Scapholeberis*, *Simocephalus*, *Ilyicriptus*, and *Macrothrix* from Anomopoda; *Holopedium* and *Sida* from Ctenopoda; and *Polyphemus* from Onychopoda). Separating *Daphnia* species from the other Cladocera resulted in very similar slopes (1.31 vs. 1.46 for *Daphnia* and non-*Daphnia* species, respectively; Table 1) that were not significantly different (ANCOVA, $F_{33,1} = 40.365$, $P < 0.0001$ and ANCOVA, $F_{33,1} = 0.982$, $P = 0.329$ for the intercept and slope, respectively). Therefore we believe that the results presented in Fig. 2b are representative of the Cladocera in general.

Discussion

Our data are consistent with the idea that the very small genome size and the high RNA:DNA ratio in cladocerans relative to copepods may reflect a reallocation of P at the cellular level. In cladocerans geared for fast growth, there is a need for maximum allocation of P to RNA to keep a high rate of protein synthesis. There are at least two prerequisites for this hypothesis: (1) the consumer's growth and reproductive rates must be chronically or at least occasionally limited

Fig. 1. (*a*) DNA content, expressed as diploid (2C) genome size in cladocerans and copepods; (*b*) phosphorus content in cladocerans and copepods; (*c*) the ratio of RNA to DNA in cladocerans and copepods; and (*d*) body phosphorus of dry mass allocated to DNA, RNA, and the sum of both nucleic acids for cladocerans and copepods. Significant differences between groups (one-way ANOVA) are denoted as follows: **, $P < 0.001$; *, $P < 0.05$; and ns, $P > 0.05$. The number of species for each group is in parentheses. Error bars are SD.
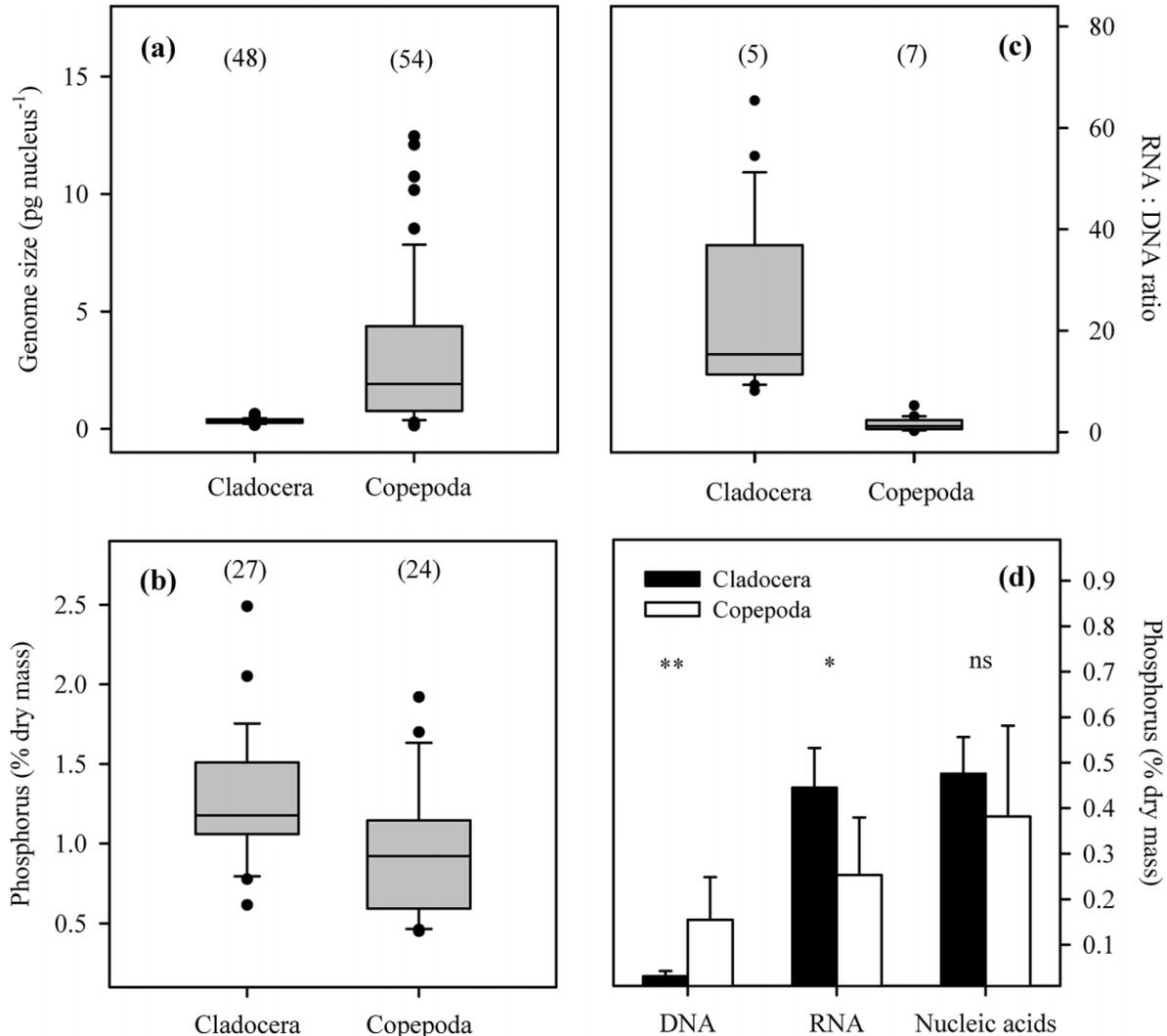
Table 1. Relationships between DNA content (diploid genome size) and body size in species of freshwater and marine calanoid copepods, freshwater cyclopoid copepods, and freshwater cladocerans.

	d.f.	F value	P value	R^2	Slope	Intercept
Both copepod orders	1, 49	43.5	<0.001	0.480	0.28	0.14
All calanoid species	1, 32	26.2	<0.001	0.466	0.42	0.01
Marine calanoid species	1, 13	10.1	0.007	0.436	0.35	0.09
Freshwater calanoid species	1, 15	10.2	0.006	0.404	0.46	–0.03
All cyclopoid species	1, 17	8.4	0.011	0.359	0.23	0.18
All cladoceran species	1, 34	56.6	<0.001	0.625	1.50	0.60
Daphnia species	1, 23	15.4	0.001	0.402	1.31	0.52
Non-*Daphnia* species	1, 9	20.5	0.002	0.690	1.46	0.56

by dietary access to P, and (2) the allocation of P from DNA to RNA must not have major fitness costs, at least not relative to the gains of increasing allocation to RNA.

With regard to the first prerequisite, there is a strong positive correlation between RNA and growth rate across a wide range of invertebrate phyla and prokaryotes (Sutcliffe 1970; Elser et al. 1996, 2003). Within the Cladocera specifically, there seems to be a consistent set of positive associations among specific P content, RNA content, and growth rate (Main et al. 1997; Gorokhova et al. 2002; Elser et al. 2003). Cladocera, and notably those with the highest P content, such as *Daphnia* spp., may frequently face P limitation

been documented, notably in high-Arctic cladoceran lineages (Beaton and Hebert 1989, 1997). Differences in cytotypes due to chromatin diminution or endopolyploidy would probably reflect interspecific more than intraspecific variability in DNA content, but clearly both of these effects might obscure the suggested allocations of P between RNA and DNA. Our cladoceran samples were mostly from temperate regions, however, with assumed diploid dominance.

Our P-allocation hypothesis is likely to be less relevant for vertebrates, for which bony structures make up a major store of P (Sterner and Elser 2002) that should readily buffer cellular needs for P. It should, however, have validity for other invertebrate taxa that may face P limitation of growth and that are characterized by considerable variation in genome size. Comparing elemental support and demands in both aquatic and terrestrial ecosystems demonstrates a strong mismatch and that P limitation of growth rates indeed could be widespread among invertebrate herbivores (Elser et al. 2000). For example, species of the family Drosophilidae, which in general have very small genomes (1C = 0.12–0.34 pg, Gregory 2008), are also likely to face dietary constraints for P (Markow et al. 1999). P limitation could also be relevant for microorganisms, for which a variety of studies have revealed remarkable abilities to streamline element demands at the biochemical level (e.g., Baudouin-Cornu et al. 2001; Ranea et al. 2005). Generally such streamlining has been attributed to the metabolic costs of maintaining and replicating "excess" DNA, but the costs in terms of P might be more relevant than the energetic costs. Intriguingly, an endemic taxon of *Bacillus* (*B. coahuilensis*) recently isolated from severely P-limited ecosystems at Cuatro Cienegas (Mexico) seems to have an extremely small genome compared with other species of *Bacillus* (Alcaraz et al. 2008).

Opportunities to test these ideas will increase steadily as more genomic information for ecologically relevant taxa becomes available. Again, we emphasize that our proposed mechanisms do not run counter to other causes of genome size variability, but given the widespread occurrence of P limitation in various organisms (Sterner and Elser 2002), we propose that it is a hypothesis worth further scrutiny.

Acknowledgments

References

Alcaraz, L.D., Olmedo, G., Bonilla, G., Cerritos, R., Hernández, G., Cruz, A., et al. 2008. The genome of *Bacillus coahuilensis* reveals adaptations essential for survival in the relic of an ancient marine environment. Proc. Natl. Acad. Sci. U.S.A. **105**: 5803–5808. doi:10.1073/pnas.0800981105.

Alonso, M. 1996. Crustacea: Branchiopoda. *In* Fauna Ibérica. Vol. 7. Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, Madrid.

Andersen, T., and Hessen, D.O. 1991. Carbon, nitrogen and phosphorus content in common crustacean zooplankton species. Limnol. Oceanogr. **36**: 807–814.

Baudouin-Cornu, P., Surdin-Kerjan, Y., Marliere, P., and Thomas, D. 2001. Molecular evolution of protein atomic composition. Science (Washington, D.C.), **293**: 297–300. doi:10.1126/science.1061052. PMID:11452124.

Beaton, M.J., and Hebert, P.D.N. 1989. Miniature genomes and endopolyploidy in cladoceran crustaceans. Genome, **32**: 1048–1053. doi:10.1139/g89-552.

Beaton, M.J., and Hebert, P.D.N. 1997. The cellular basis of divergent head morphologies in *Daphnia*. Limnol. Oceanogr. **42**: 346–356.

Beermann, S. 1977. Diminution of heterochromatic chromosomal segments in *Cyclops* (Crustacea, Copepoda). Chromosoma, **60**: 297–344. doi:10.1007/BF00292858. PMID:870296.

Brooks, J.L. 1957. The systematics of North American *Daphnia*. Mem. Conn. Acad. Arts Sci. **13**: 1–180.

Carrillo, P., Villar-Argaiz, M., and Medina-Sánchez, J.M. 2001. Relationship between N:P ratio and growth rate during the life cycle of calanoid copepods: an *in situ* measurement. J. Plankton Res. **23**: 537–547. doi:10.1093/plankt/23.5.537.

Cavalier-Smith, T. 1978. Nuclear volume control by nucleoskeletal DNA, selections for cell volume and cell growth rate and the solution of the DNA C-value paradox. J. Cell Sci. **34**: 247–278. PMID:372199.

Cavalier-Smith, T. (*Editor*). 1985. Cell volume and the evolution of eukaryotic genome size. *In* The evolution of genome size. John Wiley and Sons, Chichester. pp. 104–184.

Dawkins, R. 1976. The selfish gene. Oxford University Press, Oxford.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A., and Schampel, J.H. 1996. Organism size, life history, and N:P stoichiometry: toward a unified view of cellular and ecosystem processes. Bioscience, **46**: 674–684. doi:10.2307/1312897.

Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., et al. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature (London), **408**: 578–580. doi:10.1038/35046058. PMID:11117743.

Elser, J.J., Hayakawa, H., and Urabe, J. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology, **82**: 898–903.

Elser, J.J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., et al. 2003. Growth rate-stoichiometry couplings in diverse biota. Ecol. Lett. **6**: 936–943. doi:10.1046/j.1461-0248.2003.00518.x.

Flössner, D. 2000. Die Haplopoda und Cladocera (ohne Bosminidae) Mitteleuropas. Backhuys Publishers, Leiden.

Gorokhova, E., Dowling, T.E., Weider, L.J., Crease, T.J., and Elser, J.J. 2002. Functional and ecological significance of rDNA intergenic spacer variation in a clonal organism under divergent selection for production rate. Proc. R. Soc. Lond. B Biol. Sci. **269**: 2373–2379. doi:10.1098/rspb.2002.2145.

Gregory, T.R. 2001. Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. Biol. Rev. Camb. Philos. Soc. **76**: 65–101. doi:10.1017/S1464793100005595. PMID:11325054.

Gregory, T.R. 2008. Animal Genome Size Database [online]. Available from http://www.genomesize.com.

Gregory, T.R., Hebert, P.D.N., and Kolasa, J. 2000. Evolutionary implications of the relationship between genome size and body size in flatworms and copepods. Heredity, **84**: 201–208. doi:10.1046/j.1365-2540.2000.00661.x. PMID:10762390.

Hardie, D.C., and Hebert, P.D.N. 2004. Genome-size evolution in fishes. Can. J. Fish. Aquat. Sci. **61**: 1636–1646. doi:10.1139/f04-106.

Hebert, P.D.N., and Finston, T.L. 1993. A taxonomic reevaluation

of North American *Daphnia* (Crustacea: Cladocera). 1. The *D. similis* complex. Can. J. Zool. **71**: 908–925. doi:10.1139/z93-119.

Hebert, P.D.N., and Finston, T.J. 1996. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). 2. New species in the *Daphnia pulex* group from the south-central United States and Mexico. Can. J. Zool. **74**: 632–653. doi:10.1139/z96-073.

Hebert, P.D.N., and Finston, T.L. 1997. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). 3. The *D. catawba* complex. Can. J. Zool. **75**: 1254–1261. doi:10.1139/z97-148.

Hessen, D.O. 1992. Nutrient element limitation of zooplankton production. Am. Nat. **140**: 799–814. doi:10.1086/285441.

Hessen, D.O., Faafeng, B.A., Smith, V.H., Bakkestuen, V., and Walseng, B. 2006. Extrinsic and intrinsic controls of zooplankton diversity in lakes. Ecology, **87**: 433–443. doi:10.1890/05-0352. PMID:16637368.

Hessen, D.O., Jensen, T.C., Kyle, M., and Elser, J.J. 2007. RNA responses to N- and P-limitation; reciprocal regulation of stoichiometry and growth rate in *Brachionus*. Funct. Ecol. **21**: 956–962. doi:10.1111/j.1365-2435.2007.01306.x.

Lewis, W.M., Jr. 1985. Nutrient scarcity as an evolutionary cause of haploidy. Am. Nat. **125**: 692–701.

Lynch, M. 2007. The origins of genome architecture. Sinauer Associates, Sunderland, Mass.

Main, T.M., Dobberfuhl, D.R., and Elser, J.J. 1997. N:P stoichiometry and ontogeny of crustacean zooplankton: a test of the growth rate hypothesis. Limnol. Oceanogr. **42**: 1474–1478.

Markow, T.A., Raphael, B., Dobberfuhl, D., Breitmeyer, C.M., Elser, J.J., and Pfeiler, E. 1999. Elemental stoichiometry of *Drosophila* and their hosts. Funct. Ecol. **13**: 78–84. doi:10.1046/j.1365-2435.1999.00285.x.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. *In* A manual on methods for the assessment of secondary productivity in fresh waters. *Edited by* J.A. Downing and F.H. Rigler. Blackwell Scientific Publications, Oxford. pp. 228–265.

McKee, M.J., and Knowles, C.O. 1987. Levels of protein, RNA, DNA, glycogen and lipid during growth and development of *Daphnia magna*. Freshw. Biol. **18**: 341–351. doi:10.1111/j.1365-2427.1987.tb01318.x.

McLaren, I.A., Sevigny, J.M., and Corkett, C.J. 1988. Body sizes, development rates, and genome sizes among *Calanus* species. Hydrobiologia, **167–168**: 275–284. doi:10.1007/BF00026315.

Nardon, C., Weiss, M., Vieira, C., and Biémont, C. 2003. Variation of the genome size estimate with environmental conditions in *Drosophila melanogaster*. Cytometry A, **55A**: 43–49. doi:10.1002/cyto.a.10061.

Negrea, S. 1983. Crustacea. Vol. 4. Fasc. 12. Cladocera. *In* Fauna Republicii Socialiste Romania. Academiei Republicii Socialiste România, Bucharest.

Orgel, L.E., and Crick, F.H.C. 1980. Selfish DNA: the ultimate parasite. Nature (London), **284**: 604–607. doi:10.1038/284604a0. PMID:7366731.

Prokopowich, C.D., Gregory, T.R., and Crease, T.J. 2003. The correlation between rDNA copy number and genome size in eukaryotes. Genome, **46**: 48–50. doi:10.1139/g02-103. PMID:12669795.

Ranea, J.A., Grant, A., Thornton, J.M., and Orengo, C.A. 2005. Macroeconomic principles explain an optimal genome size in bacteria. Trends Genet. **21**: 21–25. doi:10.1016/j.tig.2004.11.014. PMID:15680509.

Rasch, E.M., and Wyngaard, G.A. 2006. Genome sizes of cyclopoid copepods (Crustacea): evidence of evolutionary constraint. Biol. J. Linn. Soc. **87**: 625–635. doi:10.1111/j.1095-8312.2006.00610.x.

Rees, D.J., Dufresne, F., Glemet, H., and Belzile, C. 2007. Amphipod genome sizes: first estimates for Arctic species reveal genomic giants. Genome, **50**: 151–158. doi:10.1139/G06-155. PMID:17546080.

Sterner, R.W. 1993. *Daphnia* growth on varying quality of *Scenedesmus* — mineral limitation of zooplankton. Ecology, **74**: 2351–2360. doi:10.2307/1939587.

Sterner, R.W., and Elser, J.J. 2002. Ecological stoichiometry. Princeton University Press, Princeton.

Sutcliffe, W.H.J. 1970. Relationship between growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Board Can. **27**: 606–609.

Ventura, M. 2006. Linking biochemical and elemental composition of freshwater and marine crustacean zooplankton. Mar. Ecol. Prog. Ser. **327**: 233–246. doi:10.3354/meps327233.

Villar-Argaiz, M., and Sterner, R.W. 2002. Life history bottlenecks in *Diaptomus clavipes* induced by phosphorus-limited algae. Limnol. Oceanogr. **47**: 1229–1233.

Vrede, T., Andersen, T., and Hessen, D.O. 1999. Phosphorus distribution in three crustacean zooplankton species. Limnol. Oceanogr. **44**: 225–229.

Wagner, M., Durbin, E., and Buckley, L. 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Mar. Ecol. Prog. Ser. **162**: 173–181. doi:10.3354/meps162173.

Wagner, M.M., Campbell, R.G., Boudreau, C.A., and Durbin, E.G. 2001. Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under different food and temperature conditions. Mar. Ecol. Prog. Ser. **221**: 185–197. doi:10.3354/meps221185.

Weider, L.J., Elser, J.J., Crease, T.J., Mateos, M., Cotner, J.B., and Markow, J.B. 2005. The functional significance of ribosomal rDNA variation: impacts on the evolutionary ecology of organisms. Annu. Rev. Ecol. Syst. **36**: 219–242. doi:10.1146/annurev.ecolsys.36.102003.152620.

Wyngaard, G.A., Rasch, E.M., Manning, N.M., Gasser, K., and Domangue, R. 2005. The relationship between genome size, development rate, and body size in copepods. Hydrobiologia, **532**: 123–137. doi:10.1007/s10750-004-9521-5.