

COMMENT

THE SMALLEST DINOFLAGELLATE GENOME IS YET TO BE FOUND: A COMMENT ON LAJEUNESSE ET AL. “*SYMBIODINIUM* (PYRRHOPHYTA) GENOME SIZES (DNA CONTENT) ARE SMALLEST AMONG DINOFLAGELLATES”¹

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LaJeunesse and colleagues (LaJeunesse et al. 2005) have recently documented small genome sizes of *Symbiodinium* and concluded that *Symbiodinium* is a dinoflagellate lineage with the smallest genome. The conclusion is inconsistent with recent discoveries of picoplanktonic dinoflagellates. The search for the smallest genome and the effort to understand the evolutionary history of dinoflagellate genome should be an area of research in the years to come, which can be greatly aided by an understanding on the current hypotheses regarding mechanisms of genome size evolution. Even the smallest dinoflagellate genome documented to date is too large to be sequenced with current technology, but sequencing of chromosomes or expressed genes of key representative species is feasible and can be very insightful for understanding genome composition and function in this important lineage of eukaryotes.

Recently, LaJeunesse and colleagues used flow cytometric analysis of fluorescently stained DNA to document the genome size of a number of *Symbiodinium* and other dinoflagellate lineages (LaJeunesse et al. 2005). Interestingly, their results show that *Symbiodinium* genomes (1.5–4 pg DNA · cell⁻¹) are the smallest among cultured dinoflagellates that have been analyzed to date (5–200 pg DNA · cell⁻¹). However, the conclusion that *Symbiodinium*'s genome is the smallest among dinoflagellates deserves some discussion.

We are far from knowing all dinoflagellates living in the vast area of the ocean. As molecular analyses in recent years have continued to reveal novel microbial eukaryotes (Díez et al. 2001, Lopez-Garcia et al. 2001, Moon-van der Staay et al. 2001, Massana et al. 2002, 2004), it is very likely that many more dinoflagellate taxa remain to be discovered. There is little reason to assume that all undiscovered dinoflagellates possess larger genomes than *Symbiodinium* does. Furthermore, the

species included in the analysis only account for a small fraction of the approximately 2000 known dinoflagellate species. Even though all the few dinoflagellate genomes that have been measured (Rizzo 1987, Veldhuis et al. 1997, Parrow and Burkholder 2002, LaJeunesse et al. 2005) are larger than 3 pg · cell⁻¹, how can one be sure that no smaller ones exist?

Apparently, the authors have deduced their conclusion from the positive correlation between genome size and cell size (Fig. 1 in LaJeunesse et al. 2005). Such a positive correlation has been demonstrated for other organisms (Gregory 2001) and the one the authors derived for dinoflagellates is reasonable. Based on this correlation, if *Symbiodinium* is the smallest dinoflagellate, its genome is likely the smallest as well. Indeed, *Symbiodinium* cell size (6–15 μm overall, 7–11 for the taxon containing the apparently smallest genome of 1.5 pg) is the smallest among the taxa analyzed in the paper, but it may not be the apparently smallest among all described dinoflagellate species. *Symbiodinium* is not the smallest dinoflagellate when uncultured dinoflagellates are also considered. Dinoflagellate 18S rDNA has been recovered in ultraplankton (<5 μm) assemblages collected from Antarctica (Lopez-Garcia et al. 2001) and picoplankton (<3 μm) from Pacific Ocean (Moon-van der Staay et al. 2001). Taxa detected in the ultraplankton appeared to be sister to *Gymnodinium* as indicated by the maximum likelihood analysis with a strong bootstrap support (99%). In the Pacific, a genetically diverse flora of dinoflagellates is evidently present. With strong bootstrap support in the Neighbor-joining analysis, the data clearly show the presence of taxa that are closely related to the parasitic dinoflagellate *Amoebophrya*. Also retrieved from the picoplankton assemblage are relatives of *Noctiluca* as well as novel lineages.

Picoplanktonic dinoflagellates do not seem to be limited to the open ocean. Recently, from surface water samples taken from the eastern Long Island Sound (Avery Point), dinoflagellates were detected from both 0.45–5 and 0.2–3 μm size fractions by PCR of genes coding for 18S rRNA, mitochondrial cytochrome *b*, and cytochrome C oxidase subunit I (S. Lin, unpublished data).

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Although LaJeunesse et al. (2005) may over generalize in their conclusion, their report poses two challenges for dinoflagellate researchers; i.e. to look into the evolution of dinoflagellate genomes and to look for the smallest dinoflagellate genome. What makes the genome of *Symbiodinium* so “small” and that of some other dinoflagellates so enormous (>200 pg)? As referred to by LaJeunesse et al. (2005), there is a diversity of hypotheses regarding the evolution of genome size, which can be grouped to two basic categories: the “adaptive” versus the “junk DNA” theories (Petrov 2001). The suggestion that the relatively uniform *Symbiodinium* genome size may have resulted from its relatively invariable cell size directly constrained by the endosymbiotic environment (LaJeunesse et al. 2005) is consistent with the “adaptive” theory and the “nucleoskeleton” notion (Cavalier-Smith 1978). However, can the endosymbiotic environment act on the genome directly and select for a small genome, as the “optimal DNA” hypothesis (Gregory 2001) implicates? It is astounding to observe how cyanobacterial genome “adapts” to the microenvironments merely in different layers in the same oceanic water column (for a review, see Fuhrman 2003). It appears that a small genome is economical for organisms living in a stable environment and larger genomes confer advantages of versatility to utilize diverse resources on those living in more complex environments (Fuhrman 2003). The small genome of *Symbiodinium*, which lives in an endosymbiotic and arguably less variable environment, is consistent with this scenario. Coincidentally, a recent investigation on mitochondrial cytochrome *b* for a number of dinoflagellates representing different taxonomic orders shows that *Symbiodinium* isolates and the benthic *Cryptocodinium cohnii* have a lower density of mRNA editing, potentially a repair mechanism of gene mutation, than planktonic dinoflagellates, and this is postulated to be related to their apparently less variable habitats (Zhang and Lin 2005).

Are the large genomes in other dinoflagellates made up by noncoding repeated DNA sequences that result from accumulation of gene mutation? It is shown that the majority of the DNA in *C. cohnii* is inactive and restriction digestion mainly produces short DNA stretches (Allen et al. 1975, Anderson et al. 1992). If this is common in dinoflagellates, the fitness benefit, either at the cellular or subcellular levels, of carrying the apparently “junk DNA” remains to be uncovered. Some of the long hidden biological functions of the classical “junk DNA” have just started to surface (Costa 2005). Furthermore, the gene content of dinoflagellate genomes may be higher than currently believed for other eukaryotes. Recent studies reveal nuclear genes that exist in numerous copies. For instance, Lee et al. (1993) reported over 1000 copies of luciferin binding protein in *Lingulodinium polyedrum*. Peridinin-chl *a* binding protein, a nuclear-encoded, chloroplast targeted gene, was estimated to have over 5000 copies in *L. polyedrum* (Le et al. 1997). In addition, proliferating cell nuclear antigen, a gene ubiquitous in eukaryotes

and archaea that normally occurs in two to three copies, has over 40 copies in *Pfiesteria piscicida* (Zhang et al. 2006) and 100 copies in *Akashiwo sanguinea* (S. Lin et al. unpublished data). Another cell cycle related gene, mitotic cyclin, appears to have over 5000 copies in *L. polyedrum* (Bertomeu and Morse 2004). Although evidence remains to be furnished with more systematic measurement of gene copies and genome composition, current data suggests a possibility that dinoflagellate genomes may have increased as a result of extensive and repetitive duplication of individual genes, chromosomes, or even the whole genome (Beam and Himes 1984). Such extensive duplication can occur multiple times and can be followed by gene losses resulting in varying genome sizes in today’s dinoflagellates. The relative contribution of multi-copy genes and noncoding repeated DNA to the large dinoflagellate genome remains to be investigated.

Whether *Symbiodinium* can be an ideal dinoflagellate model for genome sequencing should remain a question. As far as genome size is concerned, its genome, albeit small, is as large as the human genome ($\sim 3 \times 10^9$ bp), which would prevent any attempt until sequencing technology becomes drastically cheaper and throughput higher. Any hope for the near future would rest with the possibility of bringing into culture a picoplanktonic dinoflagellate whose genome is at least 10-fold smaller, if such taxon indeed exists. Alternatively, isolation and sequencing of a single megabase-sized chromosome would be a very good start toward clarifying the peculiarities of dinoflagellate nuclear biology. So far, no chromosome sequence has been reported for any dinoflagellates. In this case, a toxic species (e.g. *Alexandrium*, *Karenia*) would likely be a good candidate to attract funding required for such effort. Finally, given that many (if not all) genes occur in numerous copies in dinoflagellates, expressed sequence tag (EST) sequencing of a well-normalized cDNA library may provide insights into genome composition of a dinoflagellate, including *Symbiodinium* and others. Previous smaller-scaled EST projects have proven very helpful in addressing particular physiological or phylogenetic questions (Okamoto and Hastings 2003, Bachvaroff et al. 2004, Hackett et al. 2004, Tanikawa et al. 2004, Patron et al. 2005).

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