

"Checks and Balances" in the identification of *Symbiodinium* genetic diversity.

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The identification of genetic diversity in dinoflagellates belonging to the genus *Symbiodinium* has been the subject of numerous studies over the last decade. During this time, a variety of molecules have been utilized. Some of these loci offer a "coarse" (i.e., 18S-rDNA) view while others provide a much "finer" (i.e., DNA fingerprinting) portrait of diversity within *Symbiodinium*. The use of sequences from the ribosomal internal transcribed spacer (ITS) region has become a standard in categorizing genetic diversity of these dinoflagellates and has revealed a plethora of ecological "types" within the genus. For some of these "types", data from microsatellite loci has uncovered additional, biologically relevant, diversity. However, one cannot help to ask if this diversity is truly "real" or results from artifacts of the molecules or techniques that are utilized. Here, I discuss ways in which *Symbiodinium* ITS and microsatellite data can be "checked" for methodological and interpretation errors. These processes not only lead to a "balance" in our quantification of *Symbiodinium* diversity, but also provide novel insight into the biology of these unique organisms.

The ribosomal internal transcribed spacers (ITS)

Among eukaryotic organisms, the ribosomal DNA (rDNA) operon is organized in a similar fashion. The operon consists of three ribosomal RNAs (5.8S, 18S and 28S), each separated by external and internal transcribed spacer regions. The 5.8S rRNA is separated from the small subunit (SSU or 18S) rRNA by the first of two internal transcribed spacers (ITS1), while the large subunit (LSU or 28S) rRNA and 5.8S are separated by the second internal transcribed spacer (ITS2). Typically, these three rRNAs, as well as both the internal and external spacers, are transcribed by RNA polymerase I into a single precursor molecule, the 35-45S pre-rRNA. This molecule undergoes a series of processing steps that ultimately leads to mature and fully functional rRNAs. Mutations in the spacer regions flanking the rRNAs are known to prevent formation of the mature molecules, suggesting that they contain essential signals required for correct processing. In recent years, both ITS1 and ITS2 have been recognized as vital components of the processing steps leading to rRNA maturation; specifically, it has been emphasized that a particular secondary structure in these regions are required for correct processing (Coleman 2003). This being the case, novel *Symbiodinium* ITS sequences could potentially be validated by comparing them to established secondary structures for the genus as well as specific *Symbiodinium* clades. For this reason, the ITS2 secondary structures from members of the eight major *Symbiodinium* clades, A-H, were elucidated and compared.

The ITS2 secondary structure of Symbiodinium

In spite of large amounts of primary sequence divergence (>60%, in some cases), a nearly common ITS2 secondary structure has been recovered from representatives of all *Symbiodinium* clades (Fig. 1). This structure is consistent with the four-helix model, which has been previously described from other organisms (Coleman 2003), including some free-living dinoflagellates (Gottschling and Plotner 2004). However, among *Symbiodinium* clades B, C, F and H, a subtle, but significant, difference in secondary structure was apparent when compared to the other *Symbiodinium* clades, free-living dinoflagellates and eukaryotes in general. This structural difference was the presence of an additional stem-loop

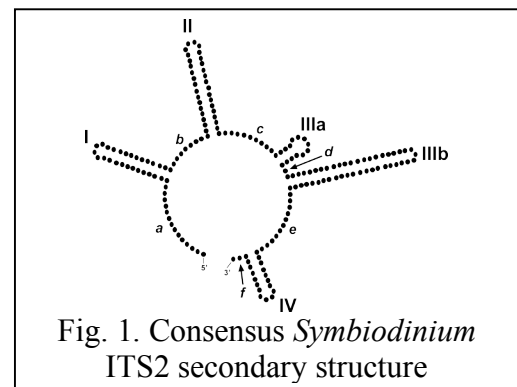


Fig. 1. Consensus *Symbiodinium* ITS2 secondary structure

the presence of an additional stem-loop

(labelled IIIa in Fig. 1), which results in a five-helix model for these clades. By using the features inherent to this ITS2 secondary structure, such as nucleotide bulges and conserved processing sites, as well as compensatory base changes found among groups of closely-related taxa, the structural skeleton for *Symbiodinium* ITS2 provided here will find use in the validation of novel sequences and optimisation of alignments for phylogenetic reconstruction.

Microsatellites

Microsatellites are simple, tandemly repeated DNA sequences elements, distributed abundantly in the genomes of virtually all organisms. For *Symbiodinium*, data from microsatellite loci (e.g., either the presence or absence of an allele, size variability between alleles or phylogenetically informative substitutions in the flanking regions adjacent to the repeat array) have demonstrated the non-representative nature of some zooxanthella cultures when compared to the populations from which they were established (Santos et al. 2001), confirmed that *Symbiodinium* spp. are haploid in the vegetative life stage (Santos and Coffroth 2003), revealed striking differentiation in *Symbiodinium* populations associated with the octocoral *Pseudopterogorgia elisabethae* across the Bahamas (Santos et al. 2003) and elucidated fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates of the Caribbean, *Symbiodinium* “type” B1 (Santos et al. 2004). However, to date, the mutational behaviour of microsatellite loci in the *Symbiodinium* genome has not been discussed. Data from two well-characterized *Symbiodinium* microsatellites suggest that a range of evolutionary processes operate on these loci.

Evolutionary patterns in Symbiodinium microsatellites

Substitutions, nucleotide insertion and deletions (indels), alterations to the repeat array structure and non-stepwise changes in repeat number have been documented from these two *Symbiodinium* microsatellites. Because the accurate estimation of population structure and relationships using microsatellite data rests in the assumption that alleles identical in state (i.e., size) have experienced a common mutational history, this discovery may complicate future studies. However, although microsatellite alleles homologous in size but resulting from different evolutionary processes (e.g., size homoplasy) were identified, this phenomenon appears to pose little problem for the interpretation of population structure if techniques capable of detecting differences in the primary sequence of microsatellite alleles are employed. Furthermore, mutations such as the ones described above are a rich source of information that complement and extend the population-level data inherent to these markers. Analysing such mutational patterns will identify the forces shaping the genome of these important organisms and provide new insight into *Symbiodinium* biology.

References

- Coleman, AW (2003). ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics*, 19: 370-375.
- Gottschling, M & Plötner, J (2004). Secondary structure models of the nuclear internal transcribed spacer regions and 5.8S rRNA in Calciodinelloideae (Peridiniaceae) and other dinoflagellates. *Nucleic Acid Research*, 32: 307-315.
- Santos, SR, & Coffroth, MA (2003). Molecular genetic evidence that dinoflagellates belonging to the genus *Symbiodinium* Freudenthal are haploid. *Biological. Bulletin*, 204: 10–20.
- Santos, SR, Taylor, DJ, & Coffroth, MA (2001). Genetic comparisons of freshly isolated vs. cultured symbiotic dinoflagellates: implications for extrapolating to the intact symbiosis. *Journal of Phycology*. 37: 900–912.
- Santos, SR, Gutiérrez-Rodríguez, C, Lasker, HR, Coffroth, MA (2003). *Symbiodinium* sp. associations in the gorgonian *Pseudopterogorgia elisabethae* in the Bahamas: high levels of genetic variability and population structure in symbiotic dinoflagellates. *Marine Biology* 143: 111–120.
- Santos, SR, Shearer, TL, Hannes, AR, & Coffroth, MA (2004). Fine-scale diversity and specificity in the most prevalent lineage of symbiotic dinoflagellates (*Symbiodinium*, Dinophyceae) of the Caribbean. *Molecular Ecology* 13: 459-469.