

Abstract

Eukaryotic organisms exhibit tremendous variability in genome size with no apparent connection to their biological complexity. Although this variation is known to correlate with numerous phenotypic traits, its evolutionary consequences remain widely unknown. This particularly applies to microalgae, where the genome size estimation is often methodologically challenging. Yet, microalgae represent a promising model group to study genome size evolution owing to their lower body complexity, short generation time and large population sizes, the latter two allowing them to quickly respond to environmental challenges. The main aim of this thesis was to enhance our understanding of genome size variation in microalgae and its evolutionary consequences. To do so, together with my co-authors, I summarized the flow cytometry (FCM) protocols used for microalgae and microorganisms possessing small genomes and addressed their limitations resulting mainly from insufficient amounts of biomass, difficulties with nuclei extraction and prominent background noise due to presence of various pigments and secondary metabolites. Further, I provided best practice recommendations that include, among others, analysing young cultures, avoiding long-term cultivation, and testing different isolation buffers and nuclei isolation techniques. Second, I introduced two new easy to use nuclei isolation protocols implementing razor blade chopping of desiccated biomass, suitable for nuclei isolation of filamentous microalgae, and bead beating for nuclei isolation of solitarily living algae. Third, to thoroughly investigate intraspecific DNA content variation and its ecophysiological consequences, we further focused on golden-brown algae (chrysophytes). We employed propidium iodide FCM to estimate nuclear DNA contents. In the most comprehensive intraspecific genome size screening conducted to date on a microalgae, we revealed a substantial genome size variability among strains of *Synura petersenii*, spanning continuously 0.97– 2.02 pg of DNA. The evolutionary mechanism generating the observed variability likely operates via gradual changes of genome size accompanied by changes in genomic GC content, such as, for example, proliferation of transposable elements. A major intraspecific DNA content variability might arise from polyploidization, as assumed in some other chrysophyte species. Alternatively, two-fold intraspecific DNA content differences might represent different life cycle stages. We revealed that the chrysophytes alternate between two ploidy stages, both of which are capable of mitotic propagation and long-term survival in cultivation. With the exception of a small increase in cell size with a higher ploidy, both life cycle stages shared the same phenotype (isomorphic) and also had highly similar genomic GC contents. Consequently, the chrysophytes have an isomorphic haploid-diploid life cycle. This was also the first report of such life cycle among unicellular algae. Interestingly, the life stage transitions in chrysophytes appear to be highly synchronized among cells, possibly due to chemical signalization. Among our investigated chrysophytes, the DNA amount was positively associated with cell size and negatively associated with growth rate. As a result, strains possessing lower DNA contents should be better colonizers or have more efficient nutrient uptake. On the other hand, strains possessing higher DNA content might better tolerate toxic environments or have higher metabolite production due to potentially increased gene dosage. Yet, these putative physiological consequences were not reflected in the geographical distribution of *S. petersenii* strains possessing various DNA contents.