Phylogenetic and structural analyses of the peculiar plastid genome of *Adenoides eludens* (Dinoflagellata)

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Abstract

Dinoflagellates are unicellular eukaryotic algae. Over half of their species are characterized by the presence of photosynthetic organelles or endosymbionts. Available data indicate that the ability to photosynthesize was acquired by dinoflagellates several times during their evolution (Bodył and Moszczyński, 2006, Eur. J. Phycol. 41: 435-48).

Among photosynthetic organelles present in dinoflagellates, the three membrane-bounded plastid containing peridinin as a main carotenoid pigment is the most enigmatic. In spite of many ultrastructural and phylogenetic studies, the evolutionary origin of this plastid is still unclear (Bodył and Moszczyński, 2006, Eur. J. Phycol. 41: 435-48). To complicate matters, sequencing projects revealed the presence of peculiar plastid genomes organized in many small circular chromosomes (0.4 -10 kb) called minicircles (Zhang et al., 1999, Nature 400: 155-9). The individual minicircles carry one, two or up to three genes, or sometimes none at all; the total number of genes reported from all peridinin plastids is less than 20. (Koumandou et al., 2004, Trends Genet. 20: 261-7). Compared to 'typical' photosynthetic plastid genomes of 120-200 kb (usually containing over 120 genes) the aforementioned features suggest dramatic genome reduction, probably by fragmentation and gene transfer to the nucleus.

Important unanswered questions remain about this peculiear genome with respecti to minicircle evolution, phylogenetic relationships among particular minicircles and the mechanism of their replication.

High-similarity regions

We analysed 9 minicircle sequences of *A. eludens* (Nelson and Green, Gene 358: 102-10). The all-against-all blastn search indicated 4.578 intra- and inter-chromosomal high-similarity regions (e < 1E-6).

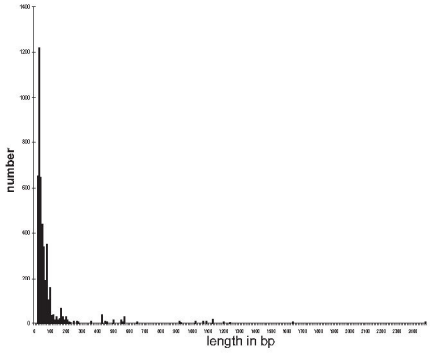


Fig. 1. Distribution of the length of high-similarity regions.

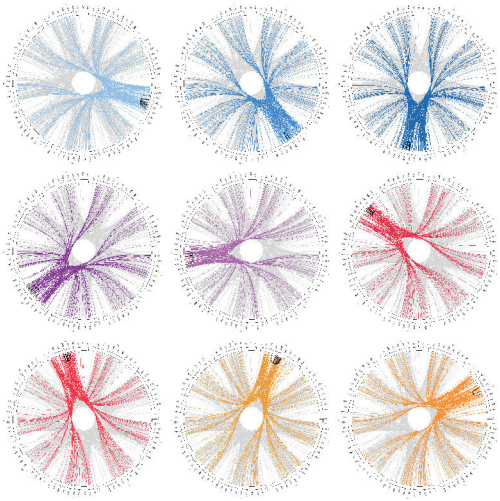


Fig. 2. The intra- and inter-chromosomal high-similarity regions. The minicircles (from AY612430 to AY612438 ordered 1-9 respectively) are plotted around the circles (length scale in bp). Black lines link intra-chromosomal high-similarity regions; grey lines link inter-chromosomal high-similarity regions (HSRs). Other color lines reflect relationships between HSRs of a given minicircle with HSRs of the remaining minicircles.

Phylogenetic analyses

We built phylogenetic nets using 4.458 HSRs including sequences of *psbA* and *psbD*.

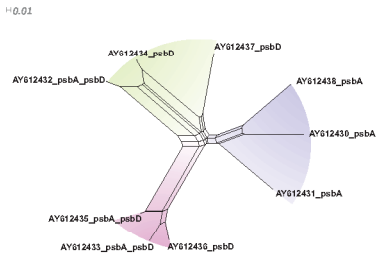


Fig. 3. Fig. 3. NeighborNet based on presence or absence of a particular HSR.

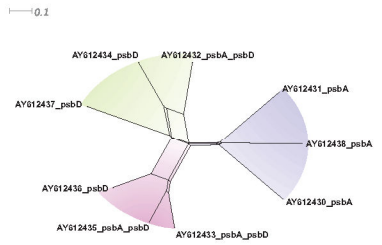


Fig. 4. NeighborNet based on distances calculated as follows:

$$d(X,Y) = 1 - \left(\frac{|X_{HSRs}| + |Y_{HSRs}|}{2\min(|X|,|Y|)} \right)^2$$

where:

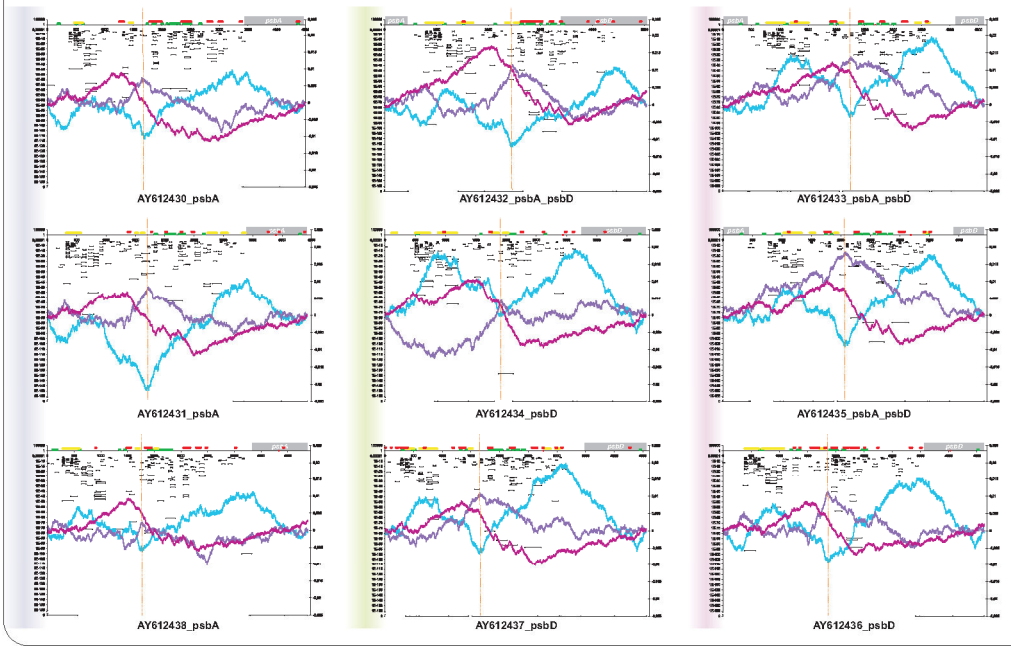
X, Y minicircles;

$|X_{HSRs}|$, $|Y_{HSRs}|$ - number of base pairs covered by the selected non-overlapping HSRs in X or Y, respectively;

|X|, |Y| - the total length of the respective minicircle.

(Auch et al., data, BMC Bioinformatics. 7: 350)

Structure and replication of minicircles



Minicircles are arranged in groups according to the NeighborNet topologies.

x-axis - sequence length in bp
left y-axis - logarithmic scale of HSRs e-values
right y-axis - scale of DNA walks

- HSRs
- inverted repeat regions
- tandem repeat regions
- palindrome regions

potential ORIs (origins of replication) were detected by the following DNA walks:

- [A-T]
- [G-C]
- [A+T]

Conclusions

Evidence for massive horizontal transfer of short, noncoding sequences and non-treelike phylogenetic signal support earlier hypotheses of frequent recombination events between minicircles.

The NeighborNet method enabled us to cluster minicircles. However, the jumbled phylogenetic signal prevented reconstruction of a detailed history of minicircle divergence and recombination events.

DNA walks detected a single, potential ORI for each minicircle.