

## Comparative plastid genomics of non-photosynthetic Orchidaceae and Ericaceae plants

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Ability for light energy transformation and storage known as photosynthesis is one of plants' principal characteristics. Organelles of symbiotic origin, called plastids, play a key role in this process. Plastids have their own genome that encodes part of photosynthesis, transcription and translation related proteins, ribosomal and transfer RNA.

Lifestyle change from photosynthetic to heterotrophic that occurs in several plant lineages causes irreversible loss of photosynthesis. This apparently leads to the relaxation of selection acting on plastid genes and could potentially lead to complete elimination of plastid genome. In spite of this, most non-photosynthetic plants retain their plastid genomes, though usually they are reduced. Ribosomal RNA genes, as well as several transfer RNA and ribosomal protein genes are universally present in all plastid genomes, even highly reduced, suggesting that translation takes place in plastids of non-photosynthetic plants. This evidences that some plastid genes have additional functions besides photosynthesis; these functions are by now unclear and can be revealed by comparative genome analysis.

Based on previous studies of plastid genome evolution in heterotrophic plants (Krause, 2008; Wicke et al., 2011), Barrett and Davis (Barrett et al., 2012) proposed a model of plastid genome degradation with five 'stages': (1) *ndh* → (2) *psa/psb*, *pet*, *rbcL*, *ycf3*, 4 → (3) *rpo* → (4) *atp* → (5) *rpl*, *rps*, *rrn*, *trn*, *accD*, *clpP*, *infA*, *matK*, *ycf1*, 2. The rationale behind this model is that in many groups, the NAD(P)H complex is the first plastid-encoded gene system to show evidence of degradation, even in some photosynthetic groups (Wicke et al., 2011; Barrett and Davis, 2012).

Second, as a result of the transition to a heterotrophic lifestyle, purifying selection is expected to be relaxed for genes involved directly in photosynthesis (stage 2), followed by degradation of the plastid-encoded RNA Polymerase complex ('PEP', or *rpo*-genes; stage 3), due to the role of the latter in transcribing plastid operons encoding many gene products with photosynthesis-related functions. The next complex hypothesized to show evidence of degradation is the ATP Synthase complex (*atp*-genes; stage 4). Although this complex is directly involved in ATP synthesis as part of the photosynthetic process, its function may remain essential in plastids other than chloroplasts (e.g., amyloplasts). The last proposed stage is the degradation of 'housekeeping' genes that play roles in basic processes of the plastid itself (Barrett et al 2014).

We used this model to study plastome degradation in Orchidaceae (Molvray et al., 2000) and Ericaceae (Braukmann, 2012), large and diverse plant families that contain many cases of independent transition to heterotrophy. *Monotropa hypopitys* (Ericaceae) and *Wulfschlaegelia aphylla* (Orchidaceae) were used as objects of our study.

We received de novo assemblies of total DNA sequence data and selected contigs of plastid origin by BLAST. Both *M. hypopitys* and *W. aphylla* have highly reduced plastid genome ~ 30 Kb in length (while in their photosynthetic relatives plastome size is ~ 150 Kb). This reduction caused by multiple gene losses and the loss of inverted repeat – large (25-30 Kb) region that is usually present in plastid genomes. We made a draft annotation by DOGMA (Wyman et al, 2004) and checked it by CPGAVAS (Liu et al, 2012).

Annotation showed lack of NAD(P)H complex, photosynthesis, PEP and ATP-synthase gene pools in *M. hypopitys* and *W. aphylla* plastomes, while majority of housekeeping genes were retained. However, the chloroplast *clpP* gene, encoding a proteolytic subunit of ATP-dependent protease, is missing in *M. hypopitys*, as well as its photosynthetic relative *Vaccinium macrocarpon* (Fajardo et al, 2013), and presents in *W. aphylla* and photosynthetic relative *Phalaenopsis aphrodite* (Chang et al 2006). This reveals lineage-specific patterns in the evolution of plastid genomes and adjusts the model of plastid gene loss proposed by Barrett and Davis.

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