

**NON-PHOTOSYNTHETIC *EUGLENA LONGA* REQUIRES
AN INTACT PLASTID GENOME FOR THE SURVIVAL
IN CONTRAST TO PHOTOSYNTHETIC *EUGLENA GRACILIS***

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Abstract

This report is a short summary of the original article by HADARIOVÁ et al. (2017) entitled “An intact plastid genome is essential for the survival of colorless *Euglena longa* but not *Euglena gracilis*”. Wild type strains of the flagellate *E. gracilis* possess three-membrane-bounded photosynthetic chloroplasts of secondary green algal origin. *E. longa* is its close relative possessing non-photosynthetic plastids with a plastid genome. The treatment of *E. gracilis* with antibacterial drugs such as ofloxacin or streptomycin leads to permanent plastid gene loss and bleaching of this flagellate without affecting its growth and viability. In contrast, the treatment of *E. longa* with ofloxacin or streptomycin, which is also accompanied by the loss of plastid genes, kills this non-photosynthetic flagellate. These results suggest that an intact plastid genome is obligatory for the survival of *E. longa* but not *E. gracilis*. A molecular mechanism of an “intermittent bleaching” was proposed for an explanation for the plastid genome reduction in *E. longa*.

Keywords: *Euglena gracilis*, *Euglena longa*, bleaching, antibacterial drugs, plastid genome reduction

The plastids of the flagellate *Euglena gracilis* are bounded by three membranes and they arose through a process of secondary endosymbiosis between a photosynthetic prasinophyte alga (HRDÁ et al. 2012; TURMEL et al. 2009) and an ancestrally heterotrophic euglenozoan host (AHMADINEJAD et al. 2007; VESTEG et al. 2010; O’NEILL et al. 2015a, b; YOSHIDA et al., 2016). The circular plastid genome of *E. gracilis* is of cyanobacterial origin and it is 143 kb in size (HALLICK et al. 1993). There are approximately 200–1000 plastid genomes copies per one *E. gracilis* cell (RAWSON & BOERMA 1976) distributed among approximately ten plastids. This plastid genome encodes one copy of each polypeptide-encoding gene, but the rRNA genes are present in

three copies. The growth of *E. gracilis* in the presence of antibacterial drugs such as streptomycin (SM) or ofloxacin (OFL) leads to the process termed bleaching – the irreversible loss of the ability to form green colonies (the loss of photosynthetic ability) (POLÓNYI et al. 1998; for review see KRAJČOVIČ et al. 2002). The influence of various antibacterial agents on plastids (as well as on bacteria) differs. OFL is a specific inhibitor of plastid (and bacterial) DNA gyrase and SM is a specific inhibitor of plastid (and bacterial) protein synthesis (SCHWARTZBACH & SCHIFF 1974).

Euglena (formerly *Astasia*) *longa* is a non-photosynthetic close relative of *E. gracilis*. It was considered to be a naturally bleached *E. gracilis* in the past (for review see BODYL 1996). This opinion challenged after the discovery of a circular 73 kb *E. longa* plastid genome (GOCKEL & HACHTEL 2000). All genes encoding photosynthesis-related protein were lost from the *E. longa* plastid genome, except for the *rbcL* gene encoding a large subunit of RuBisCo. This reduced plastid genome is transcribed (GOCKEL et al. 1994; GOCKEL & HACHTEL 2000).

The goal of the study of HADARIOVÁ et al. (2017) was to compare the influence of SM and OFL on the growth, viability and the plastid DNA content of *E. longa* and *E. gracilis*. The relative number of selected plastid genes (*rrn16*, *rrn23*, *rpl2*, *rpl16*, *rpoC2*, *tufA* and *rbcL*) in both *Euglena* species treated with SM or OFL was determined by quantitative (real-time) PCR. The treatment of *E. gracilis* with SM or OFL resulted in bleaching and the rapid decrease of the copy number of all studied plastid genes except *rpl16* without any influence on viability, growth and copy number of nuclear genes even after six weeks of antibiotic treatments. In contrast, *E. longa* was completely killed during the third week and the fifth week of SM and OFL treatment, respectively. A gradual decrease of the copy number of all studied plastid genes in *E. longa* cells had been observed during SM and OFL treatment, before the cells died. These results suggest that the loss of plastid genes (e.g. induced by antibiotics) is lethal for *E. longa* but not for *E. gracilis*.

The function of the reduced but essential plastid genome of colorless *E. longa* is currently unknown, but it is likely that it encodes at least one protein necessary for *E. longa* survival that has to be expressed. This protein(s) is (are) likely involved in an essential plastid-localized metabolic pathway(s). Such an essential metabolic pathway is unlikely to be localized solely in plastids of *E. gracilis*, but the same or similar metabolic pathway is likely rather localized in another *E. gracilis* compartment (i.e. in cytoplasm), what makes the loss of *E. gracilis* plastid genome if not the entire plastid compartment possible.

The essential non-photosynthetic *E. longa* plastid is currently not assumed to have evolved by the bleaching process. It is more likely that a selective loss of photosynthetic plastid genes (all but one) rather than a random extensive gene loss as during the bleaching process of *E. gracilis* led to the evolution of the reduced *E. longa* plastid genome. HADARIOVÁ et al. (2017) have proposed a molecular mechanism for the *E. longa* plastid genome reduction – an “intermittent bleaching” – the repeated exposures of the *E. longa* ancestor to subsaturating concentrations of reversible bleaching agents followed by the periods of growth without them. After the loss of a single photosynthetic gene, all other photosynthetic could have been lost during repeated rounds of intermittent bleaching, if they were not required for the survival. In contrast, the loss of essential non-photosynthetic plastid genes such as those involved in the expression of at least one essential plastid gene would be lethal and thus only *E. longa* cells retaining them survived.

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