

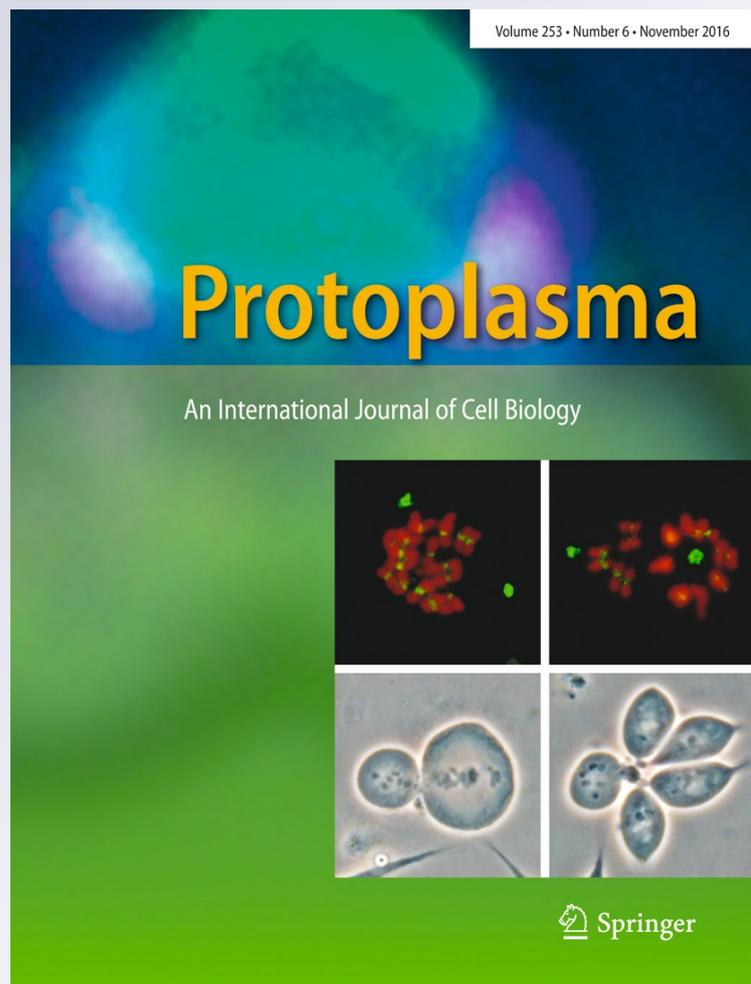
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The sexual phase of the diatom *Pseudo-nitzschia multistriata*: cytological and time-lapse cinematography characterization

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Abstract *Pseudo-nitzschia* is a thoroughly studied pennate diatom genus for ecological and biological reasons. Many species in this genus, including *Pseudo-nitzschia multistriata*, can produce domoic acid, a toxin responsible for amnesic shellfish poisoning. Physiological, phylogenetic and biological features of *P. multistriata* were studied extensively in the past. Life cycle stages, including the sexual phase, fundamental in diatoms to restore the maximum cell size and avoid miniaturization to death, have been well described for this species. *P. multistriata* is heterothallic; sexual reproduction is induced when strains of opposite mating type are mixed, and proceeds with cells producing two functionally anisogamous gametes each; however, detailed cytological information for this process is missing. By means of confocal laser scanning microscopy and nuclear staining, we followed the nuclear fate during meiosis, and using time-lapse cinematography, we timed every step of the sexual reproduction process from mate pairing to initial cell hatching. The present paper depicts cytological aspects during gametogenesis in *P. multistriata*, shedding light on the chloroplast behaviour during sexual reproduction, finely describing the timing of the sexual phases and providing reference data for further studies on the molecular control of this fundamental process.

Keywords Chloroplasts · Diatoms · Life cycle · *Pseudo-nitzschia multistriata* · Sexual reproduction · Time-lapse cinematography

Introduction

The genus *Pseudo-nitzschia* includes 45 species of marine planktonic diatoms that are important members of the phytoplankton communities in both coastal and open oceanic waters (Trainer et al. 2012; Teng et al. 2014). A considerable amount of information has been gathered in the last decades on the distribution of the different species, their physiology, toxicology and genetic diversity, making them one of the best known genera of marine phytoplankton (see reviews by Lelong et al. 2012; Trainer et al. 2012). This interest stems from the fact that some *Pseudo-nitzschia* species produce domoic acid (Mos 2001), a neurotoxin responsible for the amnesic shellfish poisoning syndrome (Pulido 2008). Since 1989, when the first paper describing the life cycle of a *Pseudo-nitzschia* species was published (Davidovich and Bates 1998), information has been gained on the life cycle features of 14 different species and 1 variety (reviewed in Lelong et al. 2012). Almost all the investigated species have a heterothallic life cycle; i.e. sexual reproduction was obtained only when strains with opposite mating type get in contact (Fig. S1). Up to now, the only documented exception is *Pseudo-nitzschia brasiliiana* Lundholm, Hasle and Fryxell, where sexual stages were observed in clonal cultures (Quijano-Scheggia et al. 2009). The basic mode of the sexual phase of the life cycle is conserved among *Pseudo-nitzschia* species (Lelong et al. 2012). Upon mixing two strains of compatible mating type and in the cell size window for sexualization, some cells align side to side and differentiate into gametangia. Two gametes are produced within each gametangium; one gametangium produces *active*

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