



Rare interspecific breeding in *Pseudo-nitzschia* (Bacillariophyceae)

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Abstract

The biological species concept defines species based on sexual incompatibility between strains and the F1 sub-viability or inviability. However, to date, there is only a limited number of studies that formally deal with sexual incompatibility in unicellular protists and hence the rigorous application of biological species concept is fuzzy in these organisms. Here, we investigated interbreeding between two species of the planktonic pennate diatom *Pseudo-nitzschia*, *P. arenysensis* and *P. pseudodelicatissima*. We observed hybridization between these two species in controlled laboratory condition. The F1 generation showed: i) low viability; ii) morphological, ultrastructural, and morphometric features that resembled those of one of the parental strains (*P. pseudodelicatissima*); iii) intermediate maximum cell size to the one observed in intraspecific sexual crosses in the parental species. Our results may suggest that interbreeding between these two species is possible although likely rare. We invite a larger body of experimental evidence in unicellular protists to assess the applicability of the biological species concept to these organisms.

Introduction

Interspecific hybridization occurs in nature (Stebbins 1950, 1959, Barton & Hewitt 1985) and it is one of the possible mechanisms through which speciation takes place (Mallet 2007). Although it is not a very common event (Mayr 1963), up to 10% of animals and 25% of plants can hybridize (Mallet 2005), with a higher percentage of hybridization in higher plants (Mallet 2005). Knowledge of interspecific hybridization in unicellular protists is scarce. Among the species with known hybridization mechanisms there are red algae (Rhodophyceae) (Destombe *et al.* 2010, Niwa *et al.* 2010), and brown algae (Phaeophyceae). In particular for the latter, hybridization has been shown both between congeneric species (Sauvageau 1909, Gard 1910, Burrows & Lodge 1953, Rice & Chapman 1985, Scott & Hardy 1994, Coyer *et al.* 2002, 2006, 2007, Coyne & Orr 2004, Zardi *et al.* 2011, Forslund & Kautsky 2013) and between species belonging to different genera (Lewis & Neushul 1995, Liptack & Druehl 2000). Evidence of interspecific hybridization in marine and freshwater diatoms is lacking except for a few laboratory based studies showing interbreeding between different varieties of the same species (*e.g.* Geitler 1958, Mann 1999, Casteleyn *et al.* 2009), and between cryptic species (Behnke *et al.* 2004, Vanormelingen *et al.* 2008). Albeit pre-zygotic barriers were investigated in the studies mentioned above, post-zygotic isolation has been studied only in cryptic or pseudocryptic diatom species (*e.g.* Behnke *et al.* 2004, Casteleyn *et al.* 2009; for a review on reproductive isolation and species boundaries please see Amato 2010a).

Pseudo-nitzschia H. Peragallo in Peragallo & Peragallo (1900: 263, 298) species co-occur in the natural habitat, and massive sexual events have been recorded in the Gulf of Naples (Holtermann *et al.* 2010, Sarno *et al.* 2010). In the same coastal system, the planktonic diatom *Pseudo-nitzschia multistriata* (Takano 1993: 39, figs. A–E) Takano (1995: 73) has been recorded to have regular sexual reproduction with a cycle of two years (D’Alelio *et al.* 2010). However, because hybrids have never been observed in nature, the existence of pre-zygotic barriers has been only hypothesized. *Pseudo-nitzschia pseudodelicatissima* (Hasle 1976: 103) G.R. Hasle (1993: 319) and *P. arenysensis* S. Quijano-Scheggia, E. Garcés, N. Lundholm in Quijano-Scheggia *et al.* (2009: 506, figs. 30–34) regularly bloom in the Gulf of Naples and in certain periods of the year co-occur (McDonald *et al.* 2007, Ruggiero *et al.* 2015). Previous mating experiments between these two species have shown no obvious interbreeding behavior (*P. pseudodelicatissima* = pse1 and *P. arenysensis* = dell in Amato *et al.* 2007). Here we provide evidence of interbreeding between these two species in controlled laboratory conditions and discuss the implication of this result for natural populations.