SCIENTIFIC REPORTS
nature research

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Sexual reproduction and genetic polymorphism within the cosmopolitan marine diatom *Pseudo-nitzschia pungens*

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Different clades belonging to the cosmopolitan marine diatom *Pseudo-nitzschia pungens* appear to be present in different oceanic environments, however, a 'hybrid zone', where populations of different clades interbreed, has also been reported. Many studies have investigated the sexual reproduction of *P. pungens*, focused on morphology and life cycle, rather than the role of sexual reproduction in mixing the genomes of their parents. We carried out crossing experiments to determine the sexual compatibility/incompatibility between different clades of *P. pungens*, and examined the genetic polymorphism in the ITS2 region. Sexual reproduction did not occur only between clades II and III under any of experimental temperature conditions. Four offspring strains were established between clade I and III successfully. Strains established from offspring were found interbreed with other offspring strains as well as viable with their parental strains. We confirmed the hybrid sequence patterns between clades I and III and found novel sequence types including polymorphic single nucleotide polymorphisms (SNPs) in the offspring strains. Our results implicate that gene exchange and mixing between different clades are still possible, and that sexual reproduction is a significant ecological strategy to maintain the genetic diversity within this diatom species.

At least 20 different species concepts have been formulated[1–3], including those based primarily on the identification of unique morphological features, those based on the sexual compatibility, and those based on phylogenetic or other evolutionary or ecological features[4]. Phytoplankton species are generally delineated on morphological characteristics, however phylogenetic studies have shown that molecular genetic data can indicate species boundaries which differ to those postulated based solely on morphological features. Those species which cannot be distinguished on morphological criteria alone are known as 'cryptic' species[5,6]. There are many cryptic or pseudo-cryptic species in plants and animals as well as micro-organisms including phytoplankton species[7–11]. Among many species concepts, an application of the biological species concept can be used to clarify species delineation in heterothallic species, as mating compatibility information can explain the discrepancies within microorganism species which are difficult to distinguish based on molecular genetic or morphological features[1,12].

Phenomena such as meiosis and crossing over, mutations, lateral gene transfer maintain genetic diversity which potentially allows species to adapt to various conditions[13]. Genetic variability can arise through several mechanisms; firstly, by migration and genetic recombination through sexual reproduction of co-occurring populations or genotypes[14,15]; secondly, by mutations[16–18]; and thirdly, by gene transfer[19,20]. Interbreeding is significant in the life and evolution of diatoms. Diatoms have a unique life-cycle in which they undergo continued vegetative cell division, resulting in cell size decrease, followed by cell size recovery through sexual reproduction[21–24].

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