

# Reproductive barriers in the diatom *Seminavis robusta* species complex and their role in species diversification

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Despite their enormous diversity and ecological importance, the understanding of diatom speciation is largely uncharted territory. Several studies showed a large cryptic species diversity in microalgae, with a large variety of reproductive systems in some species complexes. Since sexual reproduction is an obligate stage in the life cycle of most diatoms, their evolutionary success may be related to this widespread variation in reproductive systems and rapid evolution of their highly sophisticated signalling systems involved in mating. In this study, we collected a set of *Seminavis robusta* strains from the Veerse Meer and the Grevelingenmeer (the Netherlands) and the spuikom (Belgium).

Phylogenetic analysis based on *rbcL* and LSU rDNA sequences shows that these strains form three distinct cryptic lineages. Sexual reproduction can be induced with high efficiency in intra-group crosses, while inter-group mating successes drop as much as 70%. Interestingly, phylogenetic distance correlates with interbreeding capacity between mating group. This represents an interesting case in which we can dissect the contribution of different possible pre- and post-zygotic reproductive barriers between these recently diverged diatom species. We here focus on the identification of possible pre-zygotic isolating mechanisms, exploiting recent insights into the signalling mechanisms prior to mating in *S. robusta*. These include the production of a conditioning factor by both mating types which induce cell cycle arrest in the opposing mating type and the subsequent production of the pheromone L-diproline by mating type minus cells that attract mating type plus cells. Combinatorial bio-assay experiments were set up with strains of both mating types from the three mating groups.

The receptivity to conditioning factor was monitored by following cell cycle progression, while additionally for mating type minus strains the subsequent production of attraction pheromone L-diproline was measured. The role of these pre-zygotic barriers to interspecies crossing in maintaining species boundaries will be discussed.

Keywords: diatoms; speciation