Fluorescent tagging of sexual cell type identity in a marine diatom

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Diatoms are extraordinary diverse unicellular algae that play an essential role in global nutrient cycling, carbon sequestration and vastly contribute to the primary production on Earth. Unlike the organic cell walls and membranes of most eukaryotic species, diatom cell walls are made of inorganic silica, a rigid structure that limits their capacity to grow and impacts their cell division and life cycle. As a result of their peculiar cell division mechanism, the average cell size of diatom populations tends to decrease with successive vegetative divisions. Most species can only escape cell death by restoring their cell size through the production of a unique expanding cell called the auxospore during sexual reproduction. Despite their global significance, the genetic basis and regulatory mechanisms underlying their evolutionary and ecological success remain largely uncharted territory.

We performed single cell RNA sequencing (scRNA-seq) during sexual reproduction of *Cylindrotheca closterium*, a benthic and tychoplanktonic diatom species that is common on intertidal mudflats and in coastal waters in Belgium and the Netherlands. Through this approach, we identified 16 sexual cell clusters with a distinct expression profile, which cover all major steps of sexual development: mate finding, meiosis, gametogenesis, zygote formation and finally the formation of an expanding auxospore to restore maximum cell size. In addition, we report detailed in vivo molecular validation of scRNA cell cluster identity through transgenic transcriptional and translational reporter lines. Through fluorescent tagging of specific sexual cell types using the regulatory sequence of cluster-specific marker genes, we could confirm the identity of multiple scRNA-seq clusters.

Together, these results suggest several novel experimental approaches to allow a deep functional understanding of the developmental process of sexual reproduction, which is a crucial step for the survival of diatom populations. As such, our findings contribute to a better understanding of the evolutionary biology and ecological success of diatoms and can be leveraged to monitor and control natural diatom populations in the future.

Keywords
Diatoms; Cylindrotheca Closterium; Sexual Reproduction; ScRNA-seq; Transformation; Reporter Lines