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Genetic and Microscopic Evidence for Sexual Reproduction in the Centric Diatom *Skeletonema marinoi*

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This study provides microscopic and molecular evidence for sexual reproduction in the homothallic centric diatom *Skeletonema marinoi* isolated from the Baltic Sea. The species is capable of restoring cell size asexually through an auxospore-like stage. However, cells were sexualized after shifting strains from low (6 PSU) to high (16 PSU) salinity. We observed flagellate male gametes and oogonia, with diameters of 3-4 and 3.2-6.3 μm, respectively. Fertilization took place followed by the formation of round auxospores surrounded by thin siliceous incunabular scales. Auxosporulation was synchronized, and a maximum of auxospores was detected on day three following the salinity shift. The proportion of auxospores to vegetative cells ranged from 0.02 to 0.18. There was a significant correlation between auxosporulation success and inoculum cell density. At lower cell concentration (5,000 cells ml^{-1}), proportionally fewer auxospores were formed. Auxospores were formed in single strains and in crosses of strains. The proportion of auxospores differed significantly among strains and crosses of strains. Additionally, we isolated single auxospores, obtained F1 strains and performed microsatellite based pedigree analysis of parental generations and their offspring. We proved that the auxospores were formed sexually, either by inter- or by intra-strain fertilization.

Key words: Bacillariophyta; centric diatom; microsatellites; pedigree analyses; sexual reproduction; *Skeletonema*.

Introduction

The centric diatom *Skeletonema* is an important contributor to the pelagic community and often dominates phytoplankton assemblages in temperate coastal regions. It is a widespread genus except in the polar regions (Kooistra et al. 2008). Currently, thirteen species are described (Hasle and Evensen 1975, 1976; Sarno et al. 2005, 2007), but in Scandinavian water so far only one species, *Skeletonema marinoi* Sarno *et* Zingone, has been reported (Ellegaard et al. 2008; Godhe et al. 2006). In the Kattegat, Skagerrak and the Baltic Sea,

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S. marinoi is observed all year around, but is especially noteworthy during the spring bloom in February to March, when it dominates the plankton community and may reach densities of 10^4 cells per ml (Saravanan and Godhe 2010). *Skeletonema marinoi* forms benthic resting stages that are very abundant in recently deposited sediment and represent seed banks for the inoculation of future blooms (McQuoid et al. 2002). The resting stages can survive in anoxic sediment at least hundred years, and strains from different time periods can be established (Härnström et al. 2011). Further, *Skeletonema* is easy to isolate and maintain in culture (Godhe et al. 2006), and has a short mitotic generation time, approximately 1 day under laboratory conditions (Taylor et al. 2009). These features make this diatom a suitable organism for studying population genetics, dispersal ecology, microevolution, and adaptation potential under anthropogenic stressors. Progress, however, is stalled due to the limited knowledge of the reproductive cycle. Individual strains derived from vegetative division, inbreeding, or outbreeding will differ significantly in genotypes (Bengtsson 2003). This will have consequences for the allele frequencies of populations. Methods have been developed to estimate inbreeding rates and effective population size for natural populations of monoecious plants based on molecular markers (Wang et al. 2012). Given the diversity of life cycle characteristics of centric diatoms (Chepurnov et al. 2004; Kaczmarska et al. 2013), we first have to confirm their modes of reproduction.

Diatoms have a characteristic cell wall: a frustule made of biogenic silica. During the mitotic division the frustule of one of the daughter cells is smaller, and hence, the average size of the cell line decreases progressively. Restoration of cell size is an obligate part of the life cycle of most diatoms, and is typically achieved by sexual reproduction. This includes the formation of a peculiar zygote, the auxospore, and a large initial cell which is produced within the auxospore (Round et al. 1990). The induction of sexuality is possible below a species-specific cell size threshold, and only cells in their lower size range can be induced to switch from mitotic division to meiosis (Chepurnov et al. 2004). In centric diatoms, sexual reproduction is oogamous, i.e. a large non-motile egg is fertilized by a small uniflagellate sperm. Auxospores can be formed sexually (when deriving from a zygote), but occasionally apomictically, or even vegetatively (Kaczmarska et al. 2013). Sexual auxospores can be formed by allogamy, either through inbreeding or outbreeding. Strict heterothally has never been reported in centric diatoms (Chepurnov et al. 2004). Though the induction of centric diatom auxosporulation has mostly been related to endogenous factors (i.e. critical cell diameter), a role of environmental conditions as triggers of this process has been suggested. Gametogenesis and auxosporulation have been induced by light (intensity and photoperiod), temperature, salinity, and nutritional manipulations (Armbrust et al. 1990; Chepurnov et al. 2004; Drebes 1977; von Stosch 1965).

The formation of spermatogonia and oogonia, the description of the gametes, the resulting sexual auxospores, and consequential size restoration has been documented for *Skeletonema costatum* s.l. isolated from Nagasaki, south Japan (Migita 1967). Additionally, vegetative cell enlargement upon transfer of senescent cultures into fresh medium has been reported from monoclonal cultures of *S. costatum* s.l. isolated from Narrangansett Bay, USA (Gallagher 1983). Flagellate male gametes were not recorded in those strains, and cells produced by vegetative enlargement were substantially smaller than cells produced by sexual auxospores. The species diagnosis of *S. costatum* (Greville) Cleve was emended in 2005, and several new cryptic or semi-cryptic species were described (Sarno et al. 2005). Thus, it is impossible to know which species Migita (1967) and Gallagher (1983) studied, based on the illustrations and drawings provided in the papers, and considering that several *Skeletonema* species are reported from both study areas (Kooistra et al. 2008; Yamada et al. 2010).

Microsatellite markers represent an ideal tool for clarifying the sexual or asexual origin of cell lines. Microsatellites are highly polymorphic, and thus allow the identification of individual strains by unique genetic profiles. Moreover, they are inherited in a co-dominant Mendelian manner and permit the reconstruction of possible pedigrees to identify parental strains (Ellegren 2004). Centric diatoms display multiple types of sexual reproduction and asexual size restoration, which subsequently develop into an auxospore-like stage (Kaczmarska et al. 2013). Microscopic confirmation of the presence of male gametes and a microsatellite-based pedigree analysis make it possible to determine the mating system and to discriminate between homothallic inbreeding vs. outbreeding, provided that the genotypes of the parental strains are known.

The overall objective of this study was to gain a better understanding of the mating system and mechanisms involved in auxosporulation and subsequent cell enlargement of the common marine centric diatom *S. marinoi* isolated from the Baltic