Protist, Vol. 159, 73—90, January 2008
http://www.elsevier.de/protis
Published online date 25 October 2007

Protist

ORIGINAL PAPER

Genetic Divergence and Reproductive Barriers among Morphologically Heterogeneous Sympatric Clones of *Eunotia bilunaris* Sensu Lato (Bacillariophyta)

Pieter Vanormelingen[a], Victor A. Chepurnov[a], David G. Mann[b], Koen Sabbe[a], and Wim Vyverman[a,1]

[a]Laboratory for Protistology and Aquatic Ecology, Biology Department, Ghent University, Krijgslaan 281—S8, 9000 Gent, Belgium
[b]Royal Botanic Garden, Edinburgh EH3 5LR, Scotland, UK

Submitted March 30, 2007; Accepted August 3, 2007
Monitoring Editor: Marina Montresor

The study of reproductive isolation between populations, combined with estimates of genetic divergence, provides important insights into mechanisms of speciation. In this study, sixteen morphologically heterogeneous sympatric clones of *Eunotia bilunaris* sensu lato (Bacillariophyta) were brought into culture to study their phylogenetic relationships and pre- and postzygotic reproductive barriers. An ITS rDNA phylogeny was congruent with morphology and divided the clones into three groups ('slender', 'robust' and 'labile'), pointing to the presence of several species in *E. bilunaris*. Whereas most strains had a heterothallic mating system, four 'labile' clones displayed apomictic behaviour. A further 'labile' clone had a heterothallic mating behaviour, however, suggesting a very recent origin for apomixis. Despite high sequence divergence, hybridization occurred between clones belonging to different groups, but was 20—400 times less frequent than in intra-group matings. F1 hybrids had an intermediate morphology and were almost completely sterile; gamete formation was generally arrested in the early stages of meiosis I. The ITS divergence of 11.5—12.3% between the 'robust' and 'slender' clones seems to represent an upper limit of divergence in which cell pairing, gamete formation and auxosporulation are still possible but heavily reduced, and where hybrid sterility has already evolved.

Key words: apomixis; cryptic diversity; diatoms; *Eunotia*; hybridization; reproductive isolation.

Introduction

Reproductive isolation between outbreeding species can be caused by several intrinsic mechanisms, which can be divided into pre- and postzygotic barriers (Coyne and Orr 2004). Prezygotic isolation mechanisms include lack of recognition or, when incomplete, assortative mating, as a mere by-product of species divergence or as a result of selection against hybrid formation (reinforcement) when hybrids are sterile or less fit. Postzygotic mechanisms include hybrid inviability or sterility. Examining the mechanisms and extent

[1]Corresponding author; fax +32 9 264 85 99
e-mail Wim.Vyverman@ugent.be (W. Vyverman).

doi:10.1016/j.protis.2007.08.004

of reproductive isolation between (incipient) species, especially in combination with data on genetic divergence, may provide insights into the mechanisms of speciation (Edmands 2002). Although the topic has received much interest in such groups as higher plants (e.g. Moyle et al. 2004; Ramsey et al. 2003) and insects (e.g. Coyne and Orr 1997; Malausa et al. 2005), knowledge lags well behind for diatoms, the most productive and speciose group of microalgae. This is largely due to their small size and, as a consequence, more difficult experimental handling as compared to larger organisms. However, studies of sexual reproduction of diatoms in culture have shown that these difficulties can be overcome (Chepurnov et al. 2004).

Sexual reproduction is firmly integrated into the life cycles of diatoms (Round et al. 1990). Due to a peculiar mode of mitotic division in which new cell wall components are formed within the parental cell, diatoms show a steady reduction in cell size with successive vegetative divisions (the MacDonald-Pfitzer rule, e.g., Lewis 1984; Pickett-Heaps et al. 1990). After reaching a certain size threshold, the largest cell size can be restored, typically through the expansion of a specialized cell called an 'auxospore', which normally results from sexual reproduction. Sexual reproduction has repeatedly been studied in culture (Chepurnov et al. 2004) and crossing experiments for several model taxa have shown the presence of reproductive isolation between morphologically rather similar diatoms which, according to traditional morphology-based taxonomy, belong to a single species (Mann 1999). Lack of recognition seems to prevail in these crosses, but in rare cases, gamete formation or even hybridization occurs (reviewed by Mann 1999; see also Amato et al. 2007; Behnke et al. 2004). Unfortunately there has been no analysis of the frequency of gametogenesis or hybridization, nor of hybrid sterility, except in one superficially described case (p. 472 in Mann 1999). Moreover, parallel estimates of genetic divergence of the hybridizing species with molecular markers are often not available.

Eunotia bilunaris sensu lato is a good candidate for studies on reproductive isolation and semi-cryptic species diversity in diatoms (Vanormelingen et al. 2007). It is a cosmopolitan and common epiphytic diatom in oligo- to dystrophic, mainly acidic freshwater bodies (Alles et al. 1991; Krammer and Lange-Bertalot 1986—1991). Based on its wide morphological variability, a number of closely related species and intraspecific taxa of uncertain taxonomic status have been described, with numerous intergrading forms (Krammer and Lange-Bertalot 1986—1991). It can be maintained easily in culture and its life cycle shortened artificially through abrupt size reduction (Mann et al. 2003; Vanormelingen et al. 2007). The mating system is heterothallic. The sexual process is isogamous, with the first meiotic division resulting in an aborted narrow cell and a functional deep cell. The latter develops into a single gamete forming a papilla, which, by fusion with the papilla of its partner gamete, forms a copulation tube. As the gamete contents flow in, the copulation tube expands and develops into the single auxospore produced per cell-pair. Differences in morphology (width and stria density) and life cycle characteristics (perizonium structure and initial cell length) suggested that the Scottish clones studied by Mann et al. (2003) and the Australasian clones studied by Vanormelingen et al. (2007) belong to different species but this was not directly proven.

We found *E. bilunaris* populations that were heterogeneous for valve morphology in two adjacent eutrophic shallow ponds in Belgium. Genetic distances and the phylogenetic relationships between the clones were inferred using sequence analysis of the ITS rDNA region, which has been shown to be suitable for species-level phylogenetic analyses in *E. bilunaris* (Vanormelingen et al. 2007) and other diatoms (e.g. Behnke et al. 2004; Lundholm et al. 2003; Orsini et al. 2004; Zechman et al. 1994). ITS data and morphology allowed us to divide the clones into three groups (demes), one of which was capable of uniparental auxosporulation. Reproductive isolation between clones belonging to different groups was investigated, focusing on the two heterothallic demes. We provide the first detailed report on preferential mating in diatoms and hybrid sterility, in relation to data on genetic divergence.

Results

Valve Morphology

All valves analysed had a linear arcuate shape and a raphe that bent back towards the centre of the valve, both being characteristics of *Eunotia bilunaris* sensu lato (Krammer and Lange-Bertalot 1986—1991). In the natural samples, valves were 19—116 μm long (average 72 ± 19 μm), 2.65—5.89 μm wide (3.94 ± 0.54 μm) and contained 11—20 striae per 10 μm (15.1 ± 2.0 per 10 μm), which is a large morphological range but still within the limits given for the taxon by the previous authors. Ordination of valve morphometrics for the