13 Molecular genetics and the neglected art of diatomics

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ABSTRACT

Molecular systematic methods have been applied to all levels of problem in the diatoms. A possible sister group to the diatoms has been identified, the primary evolutionary radiation has been shown to be among centric diatom lineages, and the systematic positions of some problematic genera have been established. Certain structural characteristics previously used to diagnose higher taxa have been shown to exhibit homoplasy, and examination of selected complexes of diatoms has shown that cryptic and semicryptic species are probably widespread. Population genetics show that some species are panmictic over large geographical areas, while others are strongly differentiated at the population level, either geographically or temporally. Ongoing research addresses the major biogeographical question of whether diatom species are ubiquitous. On the negative side, most gene trees are disappointing because of the low statistical support for basal nodes in group-wide analyses; independent data sources (cytology, fossils) also mostly fail to discriminate between competing hypotheses of relationships. Constraints on progress include the relative poverty of culture collections and lack of multi-gene sequence data. The worst problem is the poor state of "diatomics" (holistic information about diatom species): for most diatoms, low-grade information about cell wall morphology is all that is available. Improved phylogenies are therefore often unedifying.

INTRODUCTION

Diatoms are one of the least difficult groups of plants to recognize: (1) They are unicellular or colonial protists with a special type of cell wall (Figure 13.1 [1]), consisting of two overlapping halves (thecae). (2) The thecae consist of two larger elements at opposite ends of the cell (valves) and strips (girdle bands) or scales covering the region in between (Figure 13.1 [2] and Figure 13.2 [14]). (3) The cell wall is almost always silicified. (4) During the cell cycle, cells expand along one axis only (unidirectional growth, as in the fission yeast *Schizosaccharomyces*) (Figure 13.1 [3] and Figure 13.2 [9]). (5) Cell expansion is accommodated by addition of material to the edge of the inner of the two thecae, in the central, overlapping region of the wall. (6) Cells achieve cytokinesis by simple cleavage (Figure 13.1 [3]). (7) New valves are produced within the daughter cells after cytokinesis while they are still confined within the parent cell wall (Figure 13.1 [3]). (8) Therefore, in most (but not all) diatoms, the method of cell division leads to reduction in the mean cross-sectional area and mean overall size (and often the shape) of the cells during vegetative growth. (9) Size restitution takes place via a special cell, the auxospore (Figure 13.1 [6] and Figure 13.1 [8]), which is usually zygotic. (10) Sexual reproduction (Figure 13.1 [4] through Figure 13.1 [7]) is always associated with auxosporulation. (11) Vegetative cells are diploid and the life cycle is diplontic. All of these features are unusual in plants and most are unique. A detailed account of diatom characteristics is given by Round et al. (1990).

Structurally, there are two main types of diatoms. In centric diatoms, systems of ribs and pores radiate out from a central (occasionally eccentric) ring, the "annulus" (Figure 13.1 [10], Figure 13.1 [13], and Figure 13.3 [15]), though this is sometimes difficult to discern because of extra, superposed layers of silica (Figure 13.2 [14]). In pennate diatoms, on the other hand, the valve pattern is organized bilaterally, with systems of ribs and pores arranged about a longitudinal bar, called the "sternum" (Figure 13.3 [17] and Figure 13.3 [18]), which, like the annulus, is usually but not always central. Most pennate diatoms are elongate (Figure 13.1 [1], Figure 13.3 [17], and Figure 13.3 [18]). Centric diatoms are sometimes elongate (Figure 13.2 [11] and Figure 13.2 [12]), but generally less so than pennate diatoms, and most have valves that are circular or shortly elliptical, triangular, or polygonal. Although sexual reproduction is still undocumented in several major groups of diatoms, it appears that centric diatoms are primitively and usually oogamous, with uniflagellate sperm (Figure 13.1 [5] and Figure 13.1 [6]). Pennate diatoms, on the other hand, are usually isogamous (Figure 13.1 [7]), although the gametes may be differentiated biochemically and behaviourally (e.g. Chepurnov et al., 2004). Centric and pennate diatoms were recognized as separate orders or classes for most of the 20th century (e.g. Hustedt, 1927–1966; Fritsch, 1935).

Although diatoms were discovered at the beginning of the 18th century (Anonymous, 1703), they were not studied in detail until over a century later. Throughout the subsequent history of diatom systematics, technology has been an important driver. First there was the slow perfection of optical microscopes, essentially complete by 1900 and allowing some remarkable studies of living diatoms (Lauterborn, 1896) and their shells (e.g. Hustedt, 1928). Then there was a period of some stability from 1900 until World War II, when transmission electron microscopy (TEM) became generally available (e.g. Kolbe and Gölz, 1943; Desikachary, 1952). Scanning electron microscopy (SEM) started to make an important contribution in the late 1960s (Hasle, 1968; Round, 1970), and the more widespread availability of computers in the 1980s allowed implementation of hitherto impractical methods of classification and analysis (Stoermer and Ladewski, 1982; Williams, 1985; du Buf and Bayer, 2002). The pace of change quickened recently, with the establishment of novel means of communication via the Internet (e.g. see Mann et al., 2006) and the introduction (Medlin et al., 1988, 1991) and semi-automation of molecular genetic analysis.

Almost every previous technological advance has led to improvements in our ability to discern, interpret, and describe physical structures: we now see more than we did. By contrast, molecular genetic techniques, although they characterize parts of real molecules, have an almost metaphysical outworking: the data they produce are generally not valued by taxonomists for what they are in themselves, only

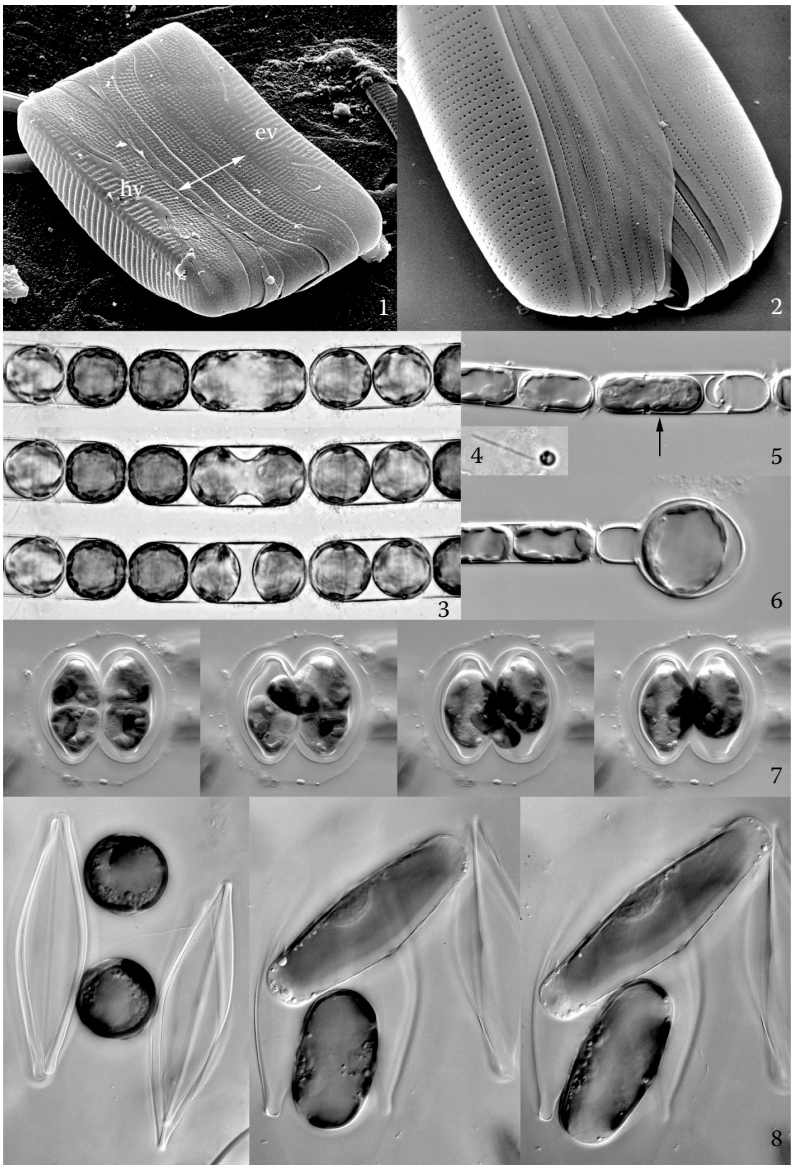

FIGURE 13.1 (1) Whole frustule of *Eunotia*, showing the two valves (hv and ev = hypovalve and epivalve) separated by sets of girdle bands (arrow). Note also the two short raphe slits on each valve (cf. 8). (2) Slightly disrupted frustule of *Parlibellus*, showing the overlap between the epitheca (left) and hypotheca, each of which consists of a valve and a set of girdle bands attached to it. (3) *Melosira*: a cell undergoing cytokinesis (after cell elongation and mitosis); the other cells in the chain are in interphase and are much shorter along the pervalvar axis. Note that pairs of cells from recent cell divisions are still contained within the parental cell wall, demonstrating the internalized cell wall formation present throughout the diatoms. (4) Uniflagellate sperm of *Actinocyclus*. (5) Differentiated auxospore mother-cell (arrow) of *Melosira* (such cells are usually oogonia in centric diatoms, but some develop into auxospores without fertilization), with an apochlorotic residual cell (at right). (6) Expanded auxospore of *Melosira*, still attached to small vegetative cells. (7) Plasmogamy in the morphologically isogamous pennate diatom *Placoneis*. (8) Development of auxospores in *Craticula*. At left, two spherical zygotes lie between the empty frustules of the gametangia. The zygotes differentiate into auxospores, which are constrained to expand along a single axis (right two photographs) through progressive formation of a perizonium of transverse silicified bands. The cells and frustules shown here and in the other plates are of moderate size for diatoms, i.e. 20–200 μm in maximum dimension.

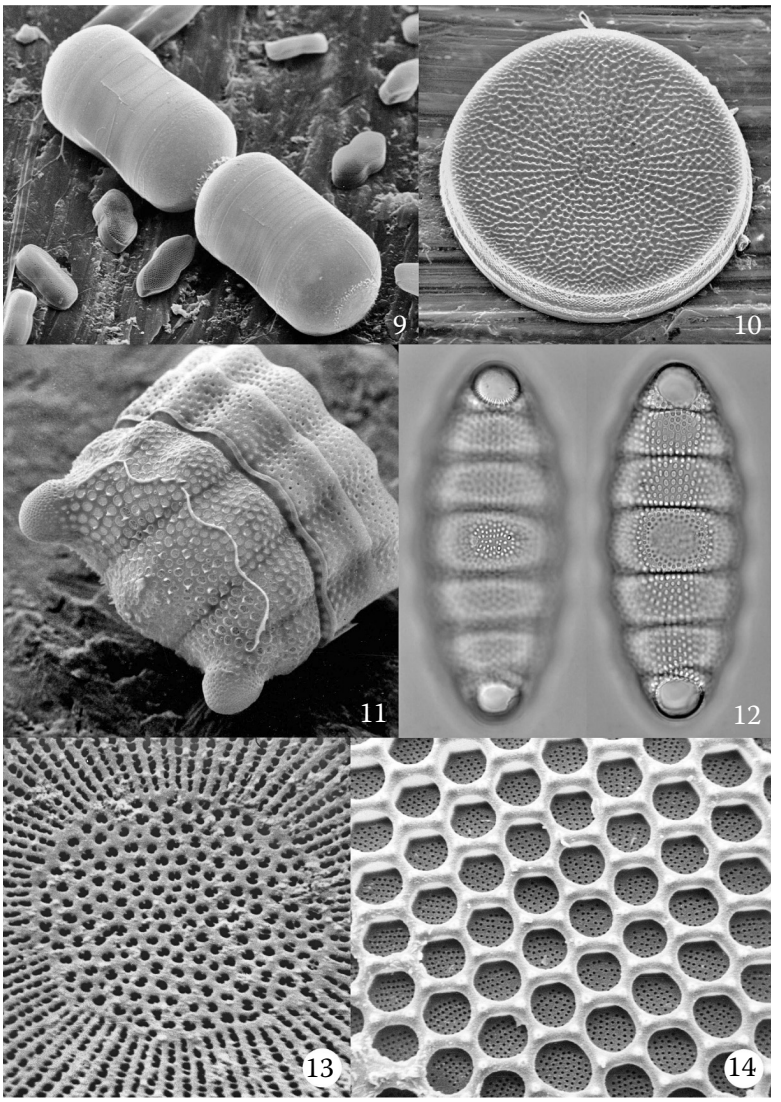

FIGURE 13.2 (9) Frustules of the radial centric diatom *Melosira*, in girdle view. (10) Frustule of the radial centric *Actinocyclus*, with radiating rows of pores. (11) The centric diatom *Biddulphia* as seen with SEM—a theca. (12) The centric diatom *Biddulphia* as seen with light microscopy—isolated valves. The valves are bipolar and lanceolate, but the striation is nevertheless radially organized. (Courtesy of Prof. F.E. Round.) (13) *Chrysanthemodiscus*: valve centre, showing ribs and lines of pores radiating from a ring (the annulus), within which pores are scattered ± evenly but irregularly. (Courtesy of Prof. F.E. Round.) (14) *Triceratium*: radiating rows of pores (an annulus can just be distinguished centrally) obscured by a superimposed system of hexagonal chambers. (Courtesy of Prof. F.E. Round.)

for what they imply about relationships between different organisms. A matrix of nucleotide or amino acid data is used to produce tree diagrams expressing our best estimates of evolution in selected genes. These "gene trees" are interpreted, with more or less care, as phylogenetic trees of organisms, and these in turn may be used as a basis for estimating the evolution and significance of morphological, cytological, reproductive, or other characters comprising the phenotype. It is rare that comparative studies of gene sequences by systematists are used to further our understanding of the genes and gene products themselves, rather than of the organisms that contain them; an exception is the analysis of

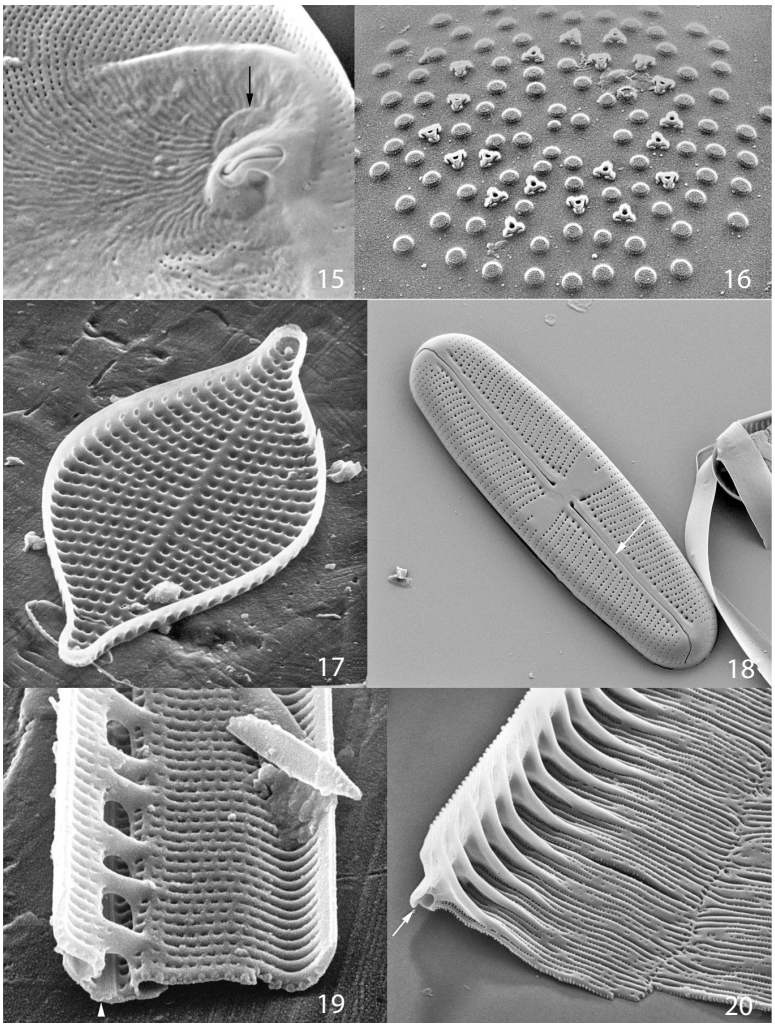

FIGURE 13.3 (15) *Guinardia*: part of the edge of the circular valve, showing the submarginal annulus (arrow). The slit-like structure is a rimoportula. (Courtesy of Prof. F.E. Round.) (16) Thalassiosirales valve: fultoportulae, each with a central pore and three satellite pores (scattered among radiating rows of valve pores, which are occluded by fine sieve-like membranes). (17) Interior of a valve of the araphid pennate diatom *Rhaphoneis*: transverse ribs and lines of pores extend out bilaterally from a principal longitudinal rib, the sternum. (18) *Sellaphora* valve, exterior, showing two raphe slits (e.g. arrow) incorporated within the sternum. (19) Interior of broken *Nitzschia* (Bacillariales) valve: a line of short, rib-like bridges of silica link the two sides of the valve together beneath the raphe (arrow). (20) Interior of broken *Cymatopleura* (Surirellales) valve, with flange-like fibulae subtending the raphe (arrow). Though similar in position, form, and presumably function, the fibulae of Bacillariales and Surirellales appear to have evolved independently.

seed-plant Rubisco by Kellogg and Juliano (1997). Thus, for the first time in the history of systematics, there is beginning to be a significant divergence between the kinds of characters we use to describe and recognize taxa and the characters that are most effective for determining relationships.

Relative to other groups of unicellular eukaryotes, diatoms have long had a sophisticated (≠ correct) taxonomy. The only groups of microalgae that can compete with them are the placoderm desmids and the armoured dinoflagellates. Diatom cell walls are easily preserved because of their silica content, and their shape, size, and patterns offer many characteristics that taxonomists can examine with the light microscope. These characteristics are remarkably constant within species and

populations, once allowance has been made for the changes (which are generally highly predictable) that accompany size reduction during the life cycle; indeed, the huge industry of diatom palaeoecology (e.g. Stoermer and Smol, 1999) would otherwise have been impossible. By the 1970s, there were enough names of genera, species, varieties, and forms to fill an eight-volume, 4600-page catalogue (VanLandingham, 1967–1978). The introduction of SEM (an interim summary was provided by Round et al., 1990) and use of protoplast (e.g. Cox, 1987, 1996) and reproductive characters (e.g. Mann, 1989) led to refinements in both classification and identification. Evolutionary schemes were produced using both informal (e.g. Simonsen, 1979) and formal (e.g. Williams, 1985; Kociolek and Stoermer, 1988) methods.

MOLECULAR APPROACHES TO DIATOM SYSTEMATICS

Molecular approaches were introduced into diatom systematics by Medlin et al. (1988, 1991) during a study of *Skeletonema* that appeared to reveal a semicryptic species previously included within *S. costatum*. The gene used by Medlin et al. was the small-subunit (SSU = 18S) component of nuclear rDNA, and Medlin has continued to work mostly with this gene.

As will be seen from Table 13.1, molecular systematics of diatoms have been dominated (roughly 3:1) by analyses of nuclear rDNA, particularly the SSU gene and partial sequences of the large-subunit (LSU) gene. The SSU gene is generally regarded as a slowly evolving region and its use in diatoms reflects this: SSU studies have been focused on high-level relationships within the diatoms, or on the relationships of diatoms to other groups of eukaryotes (Table 13.1), although available data show that there are usually a few fixed differences even between closely related species (Behnke et al., 2004). *Rbc*L is another gene that evolves relatively slowly, and the primary inspiration for recent re-classifications of angiosperm families was the large *rbc*L matrix published by Chase et al. (1993). *Rbc*L has also been used by several authors (e.g. Daugbjerg and Guillou, 2001) to examine relationships among heterokonts. We therefore started to build a large data set of diatom sequences that includes representatives of many diatom orders but focuses on the raphid group (Mann et al., 2001). This remains largely unpublished but a few sequences were used by Jones et al. (2005). Edgar and Theriot (2004) and Amato et al. (2007) are among others to make extensive use of *rbc*L in diatoms. So far, there have been no multi-gene studies of evolution within the diatoms comparable to those in green algae (e.g. Nozaki et al., 2003).

Sequence-based methods have also been used to examine relationships between closely related species, particularly in the marine planktonic diatoms *Pseudo-nitzschia* and *Skeletonema* (Table 13.1), while Edgar and Theriot (2004) have made a combined analysis of molecular and morphological data to investigate evolution in the chain-forming centric diatom *Aulacoseira*. The original motive for studying *Pseudo-nitzschia* was economic: some *Pseudo-nitzschia* species produce the neurotoxin domoic acid (e.g. Fryxell and Hasle, 2003), which causes "amnesic shellfish poisoning." It was therefore important to determine the capacity of different species to produce domoic acid and to develop fast and reliable molecular methods to identify *Pseudo-nitzschia* species, which have few characters that can be scored reliably in the light microscope. Though the induction and mechanisms of toxin production remain important research topics, we doubt whether they are now the primary justification for molecular systematics research in *Pseudo-nitzschia*, especially given that domoic acid is now known to be produced by at least one species of the huge related genus *Nitzschia* (>500 spp) and reportedly also by a species of the phylogenetically distant genus *Amphora* (Fryxell and Hasle, 2003). *Pseudo-nitzschia* has simply become an excellent model system for examining microevolution in diatoms, because of the wealth of data about molecular systematic relationships (Table 13.1), distributions (Hasle et al., 1996; Hasle and Syvertsen, 1996; Hasle, 2002), the mating system (Davidovich and Bates, 1998; Chepurnov et al., 2005; Amato et al., 2007), ultrastructure (e.g. Hasle, 1965; Lundholm et al., 2002b), and ecology (e.g. Rines et al., 2002; Cerino et al., 2005).

Until recently, sequence-based studies of inter- and intra-specific variation in *Pseudo-nitzschia*, and more recently in *Skeletonema* (Sarno et al., 2005), have relied mostly on the hypervariable region of LSU rDNA. The ITS region, whose extensive use for phylogenetic inference at or below

TABLE 13.1
Molecular Systematic Studies of Diatoms

The list includes all those we have located that deal specifically with systematic relationships among diatoms, together with some key studies of larger groups (e.g. heterokonts) in which diatom sequences are a significant component and an eclectic choice of studies not focused on diatoms that could provide a good starting point for future phylogenetic studies. The availability of the nucleotide or amino acid alignment is recorded and whether the clones used (new clones only, for analyses using a mixture of new and previously published data) were available at the time in major culture collections (e.g. CCAP, CCMP, as opposed to university or private collections); these cultures may or may not still be available (several at least are not). We assessed specification of vouchers as best we could from the text or tables; remarks like "permanent slides were made" were not accepted as a specification. "Genbank" under cultures or voucher specification indicates that the study used previously published diatom sequences available in Genbank. D, P, L, and B refer to distance, parsimony, maximum-likelihood, and Bayesian methods of analysis. Subscripts e and b indicate exhaustive analysis (for parsimony) or bootstrapping, respectively. Nucl = nucleotide-based analysis; Prot = amino acids in alignment or analysis based on translated sequence of amino acids. In describing the level of the study, we generally disregarded the outgroups.

Gene	Location of Gene	Bases in Alignment	Parsimony Informative	Number of Diatom Taxa (Accessions)	Alignment Deposited	Cultures in Public Culture Collections	Voucher Specification	Methods of Analysis	Level of Study	Ref.
SSU rDNA	Nucleus	1798	7	2 (4)	No	Yes	No	**Nucl:** P_e	*Skeletonema*	Medlin et al. (1991)
SSU rDNA	Nucleus	1565/1720	275/327	11 (11)	No	Some	No	**Nucl:** D, P_b	Heterokonts	Medlin et al. (1993)
SSU rDNA	Nucleus	1767	?	10 (12)	Printed	No	No	**Nucl:** D, P_b	Diatoms	Douglas et al. (1994)
ITS1-5.8S–ITS2 rDNA	Nucleus	725	?	4 (7)	Printed	No	No	**Nucl:** P_e	Thalassiosirales	Zechman et al. (1994)
Partial LSU rDNA	Nucleus	?	?	5 (13)	No	No	No	**Nucl:** P_b	*Pseudo-nitzschia*	Scholin et al. (1994)
Partial LSU rDNA	Nucleus	275	105	5 (5)	Printed	No	No	**Nucl:** D, P_b	Eukaryotes	Philippe et al. (1994)
Partial LSU rDNA	Nucleus	400	?	8 (8)	Printed	Yes	No	**Nucl:** D_b, P_b	Diatoms	Sorhannus et al. (1995)
SSU rDNA	Nucleus	1597	?	6 (6)	No (supply on request)	GenBank	GenBank	**Nucl:** P_b comb mol/morph	Selected eukaryotes	Saunders et al. (1995)
*tuf*A	Plastid	?740	?	3	No(available from C. Delwiche)	No	No	**Prot:** P_b **Nucl:** D_b, P_b, L_b	Life	Delwiche et al. (1995)
SSU rDNA	Nucleus	874	NA	5 (5)	No	GenBank	GenBank	**Nucl:** D_b	Selected eukaryotes	Sorhannus (1996)
SSU rDNA	Nucleus	?	?	11 (11)	?No longer available	No	No	**Nucl:** D_b	Chromalveolates	Van de Peer et al. (1996)

(*Continued*)

TABLE 13.1 (CONTINUED)
Molecular Systematic Studies of Diatoms

Gene	Location of Gene	Bases in Alignment	Parsimony Informative	Number of Diatom Taxa (Accessions)	Alignment Deposited	Cultures in Public Culture Collections	Voucher Specification	Methods of Analysis	Level of Study	Ref.
SSU rDNA	Nucleus	1711	?	29 (30)	No	GenBank	GenBank	**Nucl:** D, L	Heterokonts	Kooistra and Medlin (1996)
SSU rDNA	Nucleus	1739	528	29 (29)	No	Yes	Private collection	**Nucl:** D_b, P_b, L	Diatoms	Medlin et al. (1996a)
SSU rDNA	Nucleus	1739	528	29 (29)	No	Some	Private collection	**Nucl:** D_b, P_b, L	Diatoms	Medlin et al. (1996b)
*rbc*L	Plastid	? c. 1500	?	2 (2)	No	No	No	**Nucl:** P_b	Life	Chesnick et al. (1996)
*rbc*L–*rbc*S spacer	Plastid	43	?	2 (2)	Printed	No	No	**Nucl:** visual comparison	Eukaryotic plants	Chesnick et al. (1996)
*rbc*S	Plastid	? c. 417	?	2 (2)	No	No	No	**Nucl:** P_b	Life	Chesnick et al. (1996)
LHC[1]	Nucleus	**Prot:** 184	?	2 (2)[2]	Printed	GenBank	GenBank	**Nucl:** (D), P_b	Chromophytes	Caron et al. (1996)
Partial LSU rDNA	Nucleus	804	NA	7 (9)	Printed	?No	No	NA	NA	Miller and Scholin (1996)
SSU rDNA	Nucleus	?	?	25 (25)	No	No	No	**Nucl:** D_b	Heterokonts	Medlin et al. (1997a)
*tuf*A	Plastid	?	?	3 (3)	No	No	No	**Nucl:** D_b	Life	Medlin et al. (1997a, 2000)
16S rDNA	Plastid	?	?	16 (16)	No	No	No	**Nucl:** D_b	Life	Medlin et al. (1997a)
*rbc*L	Plastid	?	?	3 (3)	No	No	No	**Nucl:** D_b	Life	Medlin et al. (1997a, 2000)
*rbc*L	Plastid	954[3]	?	4 (4)	No	Yes	No	**Nucl:** P_b, L_b	Heterokonts	Daugbjerg and Andersen (1997)
SSU rDNA	Nucleus	?	?	28 (30)	No	No	No	**Nucl:** D_b, L_b	Heterokonts	Medlin et al. (1997b)
SSU rDNA/LSU rDNA	Nucleus	Previously published	NA	11 (11)	Previously published	GenBank	GenBank	**Nucl:** D	Diatoms	Sorhannus (1997)
SSU rDNA	Nucleus	c. 1740?	?	9 (12)	No	No	No	**Nucl:** P	Thalassiosirales	Medlin and Simon (1998)

psbV–trnR–ORF–trnM–rpl19– petF	Plastid	1881	NA	2 (2)	Printed	Yes	No	**Nucl:** (*PetF*) D_b	Life	Gueneau et al. (1998); see also Gueneau et al. (1999)
SSU rDNA	Nucleus	507	?	8 (8)	Part printed	No	No	**Nucl:** L_b	*Aulacoseira*	Shcherbakova et al. (1998)
SSU rDNA	Nucleus	1601	?	7 (7)	No	GenBank	GenBank	**Nucl:** D_b, P_b	Heterokonts	Guillou et al. (1999)
FCP	Nucleus	**Prot:** 138	?	3 (3)	Printed	Yes	No	**Prot:** L_b	Eukaryotic plants	Eppard et al. (2000)
*cox*1	Mitochondrion	**Prot:** 353	?	9 (9)	No	Yes	No	**Prot:** D_b, P_b	Chromophytes	Ehara et al. (2000a, 2000b)
*cox*1: intron reverse transcriptase/maturase	Mitochondrion	**Prot:** 146/98	NA	2 (2)	Printed	Yes	No	**Prot:** D_b	Eukaryotes	Ehara et al. (2000b)
SSU rDNA	Nucleus	1739	528	85 (85)	No	No	No	**Nucl:** D_b, P	Diatoms	Medlin et al. (2000)
16S rDNA	Plastid	?	?	12 (12)	No	No	No	**Nucl:** D	Life	Medlin et al. (2000)
TBI/Gap	Nucleus	**Prot:** ~250/~300	?	2 (2)	No	No	No	**Prot:** D_b, P_b	Life	Liaud et al. (2000)
*rbc*L	Plastid	1373	?	6 (6)	No	Some	No	**Nucl:** D_b, P_b, L_b	Heterokonts	Daugbjerg and Guillou (2001)
SSU rDNA	Nucleus	1702	236	22 (22)	No	Mostly no	No	**Nucl:** P_b, L_b	Diatoms	Beszteri et al. (2001)
5.8S + LSU/5.8S + LSU + SSU rDNA	Nucleus	? c. 3500/5300	NA	3 (3)	No	No	No	**Nucl:** D_b, (P, L)	Eukaryotes	Ben Ali et al. (2001)
SSU rDNA	Nucleus	1635	?	7 (7)	No	Yes	No	**Nucl:** D	*Thalassiosira*	Armbrust and Galindo (2001)
Partial *-tubulin*	Nucleus	671	?	1 (1)	Printed	Yes	No	**Nucl:** D	*Thalassiosira weissflogii*	Armbrust and Galindo (2001)
Partial *Sig1*	Nucleus	645	?	1 (7)[4]	Printed	Yes	No	**Nucl:** D	*Thalassiosira weissflogii*[5]	Armbrust and Galindo (2001)
SSU rDNA	Nucleus	1554	?	20 (20)	No	No	No	**Nucl:** P_b	Diatoms	Mayama and Kuriyama (2002)
Partial LSU rDNA	Nucleus	872	208	43 (56)	No	Some	No	**Nucl:** D_b, P_b, L_b	Bacillariales	Lundholm et al. (2002a)
Partial LSU rDNA	Nucleus	872	?	43 (60)	No	No	Yes (new taxa only)	**Nucl:** (D), P_b, L	Bacillariales	Lundholm et al. (2002b)
Partial LSU rDNA	Nucleus	856/570	~30/29	11 (23)	No	No	No	**Nucl:** D_b, P_b	*Pseudo-nitzschia*+	Orsini et al. (2002)

(*Continued*)

TABLE 13.1 (CONTINUED)
Molecular Systematic Studies of Diatoms

Gene	Location of Gene	Bases in Alignment	Parsimony Informative	Number of Diatom Taxa (Accessions)	Alignment Deposited	Cultures in Public Culture Collections	Voucher Specification	Methods of Analysis	Level of Study	Ref.
Partial LSU rDNA	Nucleus	872	?	45 (59)	No	No	Yes (new taxa only)	**Nucl:** P_b, L	Bacillariales	Lundholm and Moestrup (2002)
SSU rDNA	Nucleus	~1400	?	15 (15)	On request	GenBank	GenBank	**Nucl:** D_b, P_b, L	Heterokonts	Kawachi et al. (2002)
*rbc*L	Plastid	~965	?	4 (4)	On request	GenBank	GenBank	**Nucl:** D_b, P_b, L	Heterokonts	Kawachi et al. (2002; omitted codon 3)
ITS2 rDNA	Nucleus	352	?	4 (8)	No	No	No (but images in paper)	**Nucl:** matrix of similarities only	*Stephanodiscus*	Wolf et al. (2002)
Partial SSU–ITS1-5.8S–ITS2–partial LSU rDNA	Nucleus	583	?	16 (24)	No	No	Yes (new taxa only)	**Nucl:** D_b, P_b, L_b	*Pseudo-nitzschia*	Lundholm et al. (2003)
SSU rDNA	Nucleus	?~1700	412	38 (38)	No	No	No	**Nucl:** L_b	Diatoms	Kooistra et al. (2003a)
SSU rDNA	Nucleus	?~1700	NA	96 (96)	No	No	No	**Nucl:** D_b	Diatoms	Kooistra et al. (2003b)
*rpo*A	Plastid	909	314	8 (8)	No	Yes	No	**Nucl.:** D_b, P_b, L_b, B	Diatoms	Fox and Sorhannus (2003)
ITS2 rDNA	Nucleus	193	?	5 (7)	Printed	GenBank	GenBank	**Nucl:** D_b, P_b, L_b	*Stephanodiscus*	Wolf (2004)
SSU rDNA	Nucleus	1807	?	9 (9)	No[6]	GenBank	GenBank	**Nucl:** D_b, P_b, L	Eukaryotes	Kühn et al. (2004)
SSU rDNA	Nucleus	?~1800	?	?123 (123)[7]	No	Yes	No	**Nucl:** D	Diatoms	Sinninghe Damsté et al. (2004)
SSU rDNA	Nucleus	1764	764	109 (109)	No	Some	No	**Nucl:** D_b, P_b, B	Heterokonts or diatoms	Medlin and Kaczmarska (2004)
16S rDNA	Plastid	1422	526	16 (16)	No	Some	No	**Nucl:** D_b	Life	Medlin and Kaczmarska (2004)
SSU rDNA	Nucleus	1946	410	35	No	No	Yes (new taxa only)	**Nucl:** (P), L_b[8]	Diatoms	Kooistra et al. (2004)

SSU rDNA/*rbc*L/SSU rDNA + *rbc*L	Nucleus/plastid/ nucleus + plastid	?/c. 1200/?	126/99/225	15 (23)/11 (18)/15 (23)	No	Some	Yes; supplementary material on Internet	**Nucl:** P_b	*Aulacoseira*	Edgar and Theriot (2004)
SSU rDNA	Nucleus	1698	215	26 (26)[9]	Yes	Some	Yes	**Nucl:** D_b, P_b, L_b	Pennate diatoms	Behnke et al. (2004)
ITS rDNA/5.8S rDNA	Nucleus	156/155	55/10	8 (13)[10]	Yes	No	Yes	**Nucl:** D_b, P_b, L_b	*Sellaphora*	Behnke et al. (2004)
ITS1-5.8S–ITS2 /LSU rDNA	Nucleus	988/?	157/?	1 (70)/2 (10)	No	No	No	**Nucl:** D_b, (P)	*Pseudo-nitzschia*	Orsini et al. (2004)
SSU rDNA	Nucleus	—[11]	?	126 (126)	Yes	GenBank	GenBank	**Nucl:** P	Diatoms	Sorhannus (2005)
*rbc*L	Plastid	?1382	?	10 (10)[12]	No	No	Some	**Nucl:** D_b, P_b, L_b	Chromalveolates	Tamura et al. (2005)
Partial *sit* (silicon transporters genes)	Nucleus	318	NA	4 (4)[13]	Na	No	No	**Nucl:** pairwise similarity matrix	Diatoms	Sherbakova et al. (2005)
Partial SIT (silicon transporters)	Nucleus	**Prot:** ?324	?	7 (7)[14]	No	No	No	**Prot:** P_b	Pennate and multipolar centric diatoms	Thamatrakoln and Hildebrand (2005)
SSU rDNA	Nucleus	1824	297	18 (36)	Yes	Some	Yes (but new taxa only)	**Nucl:** (P)[15], L_b	*Skeletonema*	Sarno et al. (2005)
Partial LSU rDNA	Nucleus	785	122	10 (35)	Yes	Minority; most at SZN	Yes (but new taxa only)	**Nucl:** (P), L_b[16]	*Skeletonema*	Sarno et al. (2005)
Partial LSU rDNA	Nucleus	868	55	16 (55) [17]	No	No	No	**Nucl:** D_b, P_b	Bacillariales	Cerino et al. (2005)
SSU rDNA	Nucleus	~ 1770	?	10(27)[18]	No	Yes	No	**Nucl:** P_b	*Skeletonema*	Alverson and Kolnick (2005)
ITS1–5.8S–ITS2	Nucleus	982	?	2 (13)[19]	No (on request)	No (on request)	No (on request)	**Nucl:** D_b, P_b	*Cyclotella*	Beszteri et al. (2005)
Partial LSU/SSU rDNA	Nucleus	?c. 600/?c. 1800	?/?	2[20](20)/2 (8)	No (on request)	No (on request)	No (on request)	**Nucl:** D_b, P_b/—	*Cyclotella*	Beszteri et al. (2005)
HSP90/actin + alpha-tubulin + beta-tubulin +HSP90	Nucleus	**Prot:** 516/1554	?	2 (2)	No (on request)	Yes	No	**Nucl:** D_b, P_b, L_b	Eukaryotes	Harper et al. (2005)
FBA[21]	Nucleus	**Prot:** c. 400	?	3 (3)[22]	No	Some	No	**Prot:** L_b	Life	Kroth et al. (2005)
*rbc*L	Plastid	1297	?	16 (16)	No	No	Yes	**Nucl:** P_b, L_b, B	Raphid diatoms	Jones et al. (2005)
Partial LSU rDNA	Nucleus	797	?	8 (56)	No	Some	No	**Nucl:** D, L_b	*Skeletonema*	Godhe et al. (2006)
ITS1/ITS2	Nucleus	~230/~300/~694	?	1 (24)/1 (24)	No	Some	No	**Nucl:** D	*Skeletonema marinoi*	Godhe et al. (2006)

(*Continued*)

TABLE 13.1 (CONTINUED)
Molecular Systematic Studies of Diatoms

Gene	Location of Gene	Bases in Alignment	Parsimony Informative	Number of Diatom Taxa (Accessions)	Alignment Deposited	Cultures in Public Culture Collections	Voucher Specification	Methods of Analysis	Level of Study	Ref.
SSU rDNA	Nucleus	?	?	156 (156)	No	No	No	**Nucl:** D_b	Diatoms	Kooistra et al. (2006)
SSU rDNA	Nucleus	1814	343	33 (43)	No	Mostly	No	**Nucl:** D_b, P_b, L, B	Thalassiosirales	Kaczmarska et al. (2006)
ITS1–5.8S–ITS2 rDNA	Nucleus	583	?	19 (38)	No	No	Some	**Nucl:** D_b, P_b, L_b, B	*Pseudo-nitzschia*	Lundholm et al. (2006)
SSU rDNA	Nucleus	?	?	? (222)	No[23]	No	No	**Nucl:** D_b, P_b, B	Diatoms	Sims et al. (2006)
Ice-binding proteins	?Nucleus	**Prot:** 277	NA	2 (2)	Printed	No	No	NA	Life	Janech et al. (2006)
SSU rDNA/*rbc*L	Nucleus	?/?	?/?	43 (46)/11 (11)	No	No	Image	**Nucl:** D_b, P_b, L_b	Diatoms and chromalveolates	Horiguchi and Takano (2006)
SIT[24]	Nucleus	**Prot:** 615	NA	10 (11)	Printed	Some	No	**Nucl:** L_b	Diatoms	Thamatrakoln et al. (2006)
SIG1	Nucleus	**Prot:** 197	NA	4 (64)	Yes	GenBank	GenBank	NA	*Thalassiosira*	Sorhannus and Kosakovsky Pond (2006)
SSU rDNA	Nucleus	1465	?	7 (7)	No	GenBank	GenBank	**Nucl:** L, B	Life	Berney and Pawlowski (2006)
SSU rDNA	Nucleus	1519	NA	32 (32)	No	GenBank	GenBank	**Nucl:** D_b	Heterokonts	Cavalier-Smith and Chao (2006)
SSU rDNA/*rbc*L	Nucleus/ plastid	1345/1206	21/14	11 (11)	No	?Yes	Image	**Nucl:** D_b, P_b, L_b	Diatoms	Jung et al. (2006)
SSU rDNA	Nucleus	752	2034	c. 155 (189)	Yes	GenBank	GenBank	**Nucl:** P_b, L, B	Diatoms	Alverson et al. (2006)
ITS1–5.8S–ITS2	Nucleus	~720	?	1 (7)	No	No	± Yes	**Nucl:** D_b, P_b, L_b	*Eunotia bilunaris*	Vanormelingen et al. (2007)
Partial LSU rDNA	Nucleus	?c. 870	?	23 (25)	No	Some	Yes and images	**Nucl:** D_b, P_b, L_b	Bacillariaceae	Trobajo et al. (2006)
Partial LSU rDNA/ITS1–5.8S–ITS2 rDNA/*rbc*L	Nucleus and plastid	?/?/?	?	8 (92/61/78)	No	No	No	**Nucl:** L_b	*Pseudo-nitzschia*	Amato et al. (2007)

[1] Coding for fucoxanthin chlorophyll a/c binding proteins.
[2] Eleven intraclonal variants (9 in *Phaeodactylum*, 2 in *Odontella*).
[3] First and second codon positions only.
[4] Several copies of the *Sig1* gene with different sequences are present within clonal isolates of *T. weissflogii*; data for 40 intraclonal gene copies were given by Armbrust and Galindo (2001).
[5] Comparative data were tabulated for two other *Thalassiosira* species.
[6] Sequences from the ARB database.
[7] It is unclear whether divergent sequences said to be of the same taxon (e.g. *Rhizosolenia setigera*, *Cylindrotheca/Nitzschia closterium*) reflect inaccurate identification, faulty taxonomy, sequencing errors, or alignment inconsistencies.
[8] Parsimony data not shown.
[9] Demes have been treated as taxa.
[10] Behnke et al. (2004) sequenced a total of 23 clonal and intraclonal rDNA variants, but only 13 were used in their phylogenetic analysis.
[11] The sequences were derived from the aligned SSU rDNA data in the European ribosomal RNA database (www.psb.ugent.be/rRNA/index.html).
[12] Includes three diatom endosymbionts of dinoflagellates.
[13] Five different *sit* sequences included for *Cylindrotheca fusiformis*.
[14] Five different *sit* sequences included for *Cylindrotheca fusiformis* and three for *Thalassiosira pseudonana*.
[15] Parsimony data not shown.
[16] Parsimony data not shown.
[17] The named taxa correspond particularly poorly to the clades evident in the tree.
[18] Because of intraclonal variation, a total of 60 different SSU sequences were analysed.
[19] Forty-six different gene sequences recorded among and within diatom clones.
[20] Twelve different gene sequences recorded among and within diatom clones.
[21] Coding for fructose-1,6-bisphosphate aldolases.
[22] Several genes in *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, producing aldolases targeted to different cellular compartments.
[23] Sequences from the ARB database.
[24] Twenty-six different *sit* genes were isolated from ten species.

the genus level in angiosperms has been critically reviewed by Álvarez and Wendel (2003), was first used in diatoms as early as 1994 (Zechman et al., 1994), but relatively little for the next 10 years (Table 13.1). Behnke et al. (2004) used ITS sequences to examine relationships in the *Sellaphora pupula* species complex, demonstrating multiple divergent copies of ITS rDNA within single clones of *Sellaphora* species and extreme difficulty in aligning both ITS1 and ITS2 among different demes (we use "deme" to refer to a group of individuals, or a population, or a group of populations of a specified taxon that share particular phenotypic, genotypic, reproductive, ecological, or other characteristics, in a slight modification of the principles explained in detail by Gilmour and Heslop-Harrison, 1954). Some of the *Sellaphora* demes have subsequently been described as taxonomic species by Mann et al. (2004). Vanormelingen et al. (2007) have also detected intra-isolate and among-isolate ITS variation in *Eunotia bilunaris*. In *Sellaphora*, *Pseudo-nitzschia*, and *Eunotia*, ITS relationships have been compared not only with morphological variation but also with reproductive compatibility (Behnke et al., 2004; Amato et al., 2007; Vanormelingen et al., 2007). Amato et al. (2007) have evaluated how well four genetic markers (LSU, ITS1 and ITS2 rDNA, and *rbc*L) discriminate between species in the *Pseudo-nitzschia delicatissima/P. pseudodelicatissima* complex: ITS2 variation showed the best concordance with reproductive compatibility and morphology.

The impact of molecular methods on diatom systematics will be discussed in two sections, dealing in turn with supra- and infraspecific variation. Together with many others since the Modern Synthesis (e.g. Mayr, 1942) and in accordance with the arguments summarized recently by Coyne and Orr (2004), we regard species as real, not constructed more or less arbitrarily by the human mind. Their boundaries lie at the fuzzy interface between reticulate and hierarchical relationships (see also Mann, 1999), and the process by which new species are formed (speciation) is associated with restriction and loss of gene flow (reproductive isolation). According to this model, asexual organisms do not exist as species in the same way that sexual organisms do. However, available evidence indicates that diatoms are predominantly sexual organisms with the capacity to outbreed (e.g. Chepurnov et al., 2004).

SUPRA-SPECIFIC RELATIONSHIPS

The origins of diatoms

It is difficult to make an impartial assessment of the contribution molecular analyses have made to higher-level diatom systematics. Confronted with robust, well-supported conclusions from molecular studies, cynics will say that they knew it all anyway. If the results are even slightly equivocal, sceptics will say that they are totally unconvinced. Enthusiasts take hints of bootstrap support as a justification for extravagant scenario building. The ambitious may manipulate history to imply that systematics began with the invention of the polymerase chain reaction (PCR) machine. Of course, in this paper we will try to be scrupulously fair.

As a single entity recognized at the class or divisional level, the diatoms have emerged from almost two decades of molecular research as they entered it: as a single well-defined group. No organisms regarded previously as diatoms have been found not to be diatoms, and the only "unconventional" diatoms that have been added to the group are the shell-less endosymbionts of some dinoflagellates (e.g. Tamura et al., 2005; Horiguchi and Takano, 2006). Most of these endosymbiotic diatoms seem to have been derived from the Bacillariaceae, a group of fibulate raphid diatoms that arose relatively late in diatom evolution (e.g. Medlin et al., 2000) but existed by the upper Eocene, approximately 38 Mya (Schrader, 1969). However, *Peridinium quinquecorne* has apparently jettisoned its pennate symbiont in favour of a centric diatom (*Chaetoceros*) (Horiguchi and Takano, 2006). The diatoms have been shown to be a natural group, but given the combination of characters that almost all diatoms possess (our opening paragraph), this is not a surprise.

The most fundamental question remaining, therefore, is where did this remarkably well-defined group come from, and how did they acquire their trademark characteristics? Molecular systematics have not brought us the answer. Transmission electron microscopy of flagella (present in the sperm of centric diatoms) and chloroplast structure, carbohydrate storage (as β-1,3–linked glucans), and chloroplast pigments (possession of *c*-type chlorophylls, fucoxanthin, diatoxanthin, and diadinoxanthin) had shown long before 1990 that diatoms belong to the heterokonts, together with brown algae, chrysophytes, xanthophytes, eustigmatophytes, oomycetes, and some other classes of autotrophic and heterotrophic protists (van den Hoek et al., 1995). However, ultrastructure and pigmentation did not tell us how different heterokont groups are related to each other, or how they are related to other autotrophic protists with secondary endosymbionts derived from red algae, viz. the Haptophyta, Cryptophyta, and Dinophyta. These questions remain largely unanswered, despite the introduction of molecular methods (e.g. Goertzen and Theriot, 2003; Harper and Keeling, 2003; Harper et al., 2005) and the discovery of several previously unrecognized heterokont classes (e.g. Andersen et al., 1998; Kawachi et al., 2002). The sequencing of the complete nuclear, plastid, and mitochondrial genomes of *Thalassiosira pseudonana* (Armbrust et al., 2004) and the earlier sequencing of the *Odontella* plastid genome (Kowallik et al., 1995) provide almost limitless opportunities for using molecular systematics to examine relationships between diatoms and other organisms (e.g. Miyagishima et al., 2004; Vinogradov et al., 2005; Kroth et al., 2005; Foth et al., 2006), but there are currently few comparable data for other autotrophic heterokonts: there are no other plastid genomes besides *Odontella* and the only other nuclear genome is from the diatom *Phaeodactylum* (Scala et al., 2002; Maheshwari et al., 2005). However, the situation will soon change dramatically, with the sequencing of the genomes of *Aureococcus*, *Ectocarpus*, *Ochromonas*, and two other diatoms: *Pseudo-nitzschia* and *Fragilariopsis*.

There is just one well-supported new conclusion to be drawn from molecular data about the origins of diatoms: thanks to Guillou et al. (1999) and Daugbjerg and Guillou (2001), we know that one group of heterokonts, the Bolidophyceae, is particularly closely related to diatoms. Unfortunately, this discovery makes scarcely any contribution to understanding the evolution of diatom characteristics, because the known bolidophytes are tiny picoplanktonic flagellates that have no walls, are not known to produce any silicified structures or to metabolize silica, and seem to share no morphological or cytological characteristics with diatoms that are not also shared with other heterokonts. Their ploidy and life cycles are unknown (though it might be expected that such organisms would be haploid, given selection for extreme small size and low nutrient quotas). Mann and Marchant (1989) suggested that the closest relatives of the diatoms are the parmophytes, which are another group of picoplanktonic autotrophs that appear to be heterokonts and, unlike the bolidophytes, produce multipartite, patterned walls of silica that resemble diatom frustules in several ways. Unfortunately, no-one with access to parmophyte material has obtained any molecular sequence data (although attempts to culture parmophytes have failed, it would surely be possible to get sequences from environmental samples). Conceivably, parmophytes are resting stages in the life cycles of bolidophytes.

Evolution within the diatoms

Several papers have been published recently that review the contributions of molecular systematics to our understanding of relationships *within* the diatoms (Kooistra et al., 2003b; Medlin and Kaczmarska, 2004; Alverson and Theriot, 2005; Kooistra et al., 2006; Sims et al., 2006; Alverson et al., 2006), and we will not attempt to cover all the same ground. Instead, we will highlight some notable successes and then concentrate on what we do not know and the obstacles to further progress. So, first, what do we know and is it a surprise?

1. The "centric" group is paraphyletic, but there is a monophyletic group comprising most diatoms traditionally considered to be pennates (but see Kooistra et al., 2003a), specifically, those diatoms possessing a sternum. These features are evident in almost all trees

that contain representatives of several centric and pennate orders (an exception is the analysis by Van de Peer et al. [1996], but this contained only 11 diatoms), whatever the gene used—nuclear (e.g. Medlin and Kaczmarska, 2004), plastidial (e.g. Fox and Sorhannus, 2003), or mitochondrial (Ehara et al., 2000a). But did we not know this already? Well, no, we did not. Literature searches reveal remarkably few discussions of diatom evolution, and these few express even fewer strong opinions concerning the relationship between centrics and pennates. Fritsch noted that the diatoms "appear as a sharply circumscribed group of rather highly evolved forms which afford few points of vantage either for tracing of their phylogeny or of their affinities with other groups of Algae" (1935, p. 564), though he noted that fossil evidence suggested that the centrics are older than the pennates (p. 642). Round and Crawford acknowledged that fossil evidence "certainly points to a morphological sequence, centric → araphid → raphid" (1984), but considered that the fossil record was severely biased by selective preservation and loss of earlier material through erosion and re-working of deposits (Round and Crawford, 1981). They argued for a very early, Precambrian origin of diatoms and a rapid diversification of major groups, including both centrics and pennates, from a pool of ancestral diatoms (Round and Crawford, 1981; Round, 1981). So, an origin of pennate diatoms from centrics was not universally accepted before the advent of molecular systematics. Simonsen, however, was quite definite that pennate diatoms most likely developed "at some time of the early Tertiary or late Cretaceous, and they must have developed from the Centrales … but from which … we cannot tell. The Pennales were suddenly simply there" (1972). Simonsen (1979) later amplified and revised his views and summarized them in a tree that shows the early diversification of diatoms to be wholly among centric lineages; the pennate diatoms are shown as evolving from the Eupodiscaceae, a group of centric diatoms with elliptical, elongate, or multiangular valves. However, although this tree was undoubtedly based on great personal knowledge of diatom morphology and fossils, it is difficult to work out from Simonsen's descriptive text how he arrived at his conclusions.

2. The fossil record appears to be less misleading than Round and Crawford (1981) thought. Judging by the branching order in gene trees (poorly supported though many of these are), which agrees reasonably well with the first appearance of major groups in fossil deposits (Sims et al., 2006), and by molecular clock calculations (Kooistra and Medlin, 1996; Medlin et al., 1996a, 1997a, 1997b), the diatoms arose and diversified in the Mesozoic, as the fossils suggest, rather than in the Precambrian (see also Berney and Pawlowski, 2006). Nevertheless, the fossil record is certainly deficient. Using geochemical markers (highly branched C_{30} isoprenoid alkenes) that are apparently specific for *Rhizosolenia* among extant diatoms, Sinninghe Damsté et al. (2004) detected the rhizosolenid lineage in 91.5 Mya deposits, whereas the first preserved frustules of the group appear nearly 20 My later. Likewise, the earliest undoubted raphid diatoms recorded so far are from the Palaeocene and appear to be *Lyrella* species (Strel'nikova, 1992, where they are recorded as "*Navicula*"), but *rbc*L and SSU rDNA trees show that *Lyrella* is not a basal lineage within the raphid group (Jones et al., 2005).
3. Valve shape has been shown to have rather more significance than seemed to be the case 20 years ago, during the heyday of SEM studies. SEM revealed that groupings based on valve shape and symmetry often conflicted with those based on valve ultrastructure, and when extra data sets were introduced (e.g. cytological, reproductive), they tended to support ultrastructure-based classifications. For example, SEM studies of the asymmetrical diatom *Amphora* revealed several species that had valve and raphe structure like those of bilaterally symmetrical *Navicula*, and the chloroplasts and auxosporulation were also *Navicula*-like. These species were therefore reclassified into the Naviculaceae, as the genus *Seminavis* (Round et al., 1990; Danielidis and Mann, 2002; Chepurnov et al., 2002). Molecular data have confirmed that *Seminavis* is close to or within *Navicula* and

other shape-groups (e.g. *Gomphonema*) have also proved artificial (e.g. see the trees of Medlin and Kaczmarska, 2004, and Jones et al., 2005, based on SSU rDNA and *rbc*L, respectively). However, molecular approaches have provided evidence that some aspects of shape do not vary so capriciously and instead reflect major evolutionary events (Kaczmarska et al., 2001; see also Alverson et al., 2006). Thus, the major clades of centric diatoms revealed by molecular data are generally either "radial" or "multipolar" (Kooistra et al., 2003b; Medlin and Kaczmarska, 2004; Alverson and Theriot 2005), i.e. they either have circular valves (Figure 13.2 [9] and Figure 13.2 [10]), or valves that are elliptical, elongate, triangular, or multiangular (Figure 13.2 [11] and Figure 13.2 [12]). This seems to reflect a significant difference in the way the auxospores develop (von Stosch, 1982; Mann, 1994), although all generalizations are severely compromised by lack of data. In radial centrics, expansion is generally isodiametric (Figure 13.1 [6]), because the auxospore wall is homogeneous and either wholly organic or composed of an organic matrix in which small silica scales are embedded. In multipolar centric diatoms and pennate diatoms, on the other hand, auxospore expansion is accompanied by the formation of a system of silica bands (referred to as a properizonium in centric diatoms and as a perizonium in pennate diatoms, though they seem to be homologous structures), which constrain expansion to two, three, or more "soft spots" (Figure 13.1 [8]), producing bi- to multipolar shapes (Mann, 1994; Kaczmarska et al., 2001).

4. The Thalassiosirales, a group uniquely characterized by possession of special chitin-secreting organelles (fultoportulae = strutted processes: Figure 13.2 [16]), are not a basal group of centric diatoms, as previously thought. Thalassiosirales usually have circular valves, and Simonsen (1972, 1979) considered them to belong close to the Melosiraceae, a group of predominantly chain-forming "radial" centrics. Instead, they appear to have arisen from clades of elongate or multiangular ("multipolar") centric diatoms (Medlin et al., 1996a, 2000; Medlin and Kaczmarska, 2004). This implies that they have acquired circular valve morphologies secondarily, through loss of properizonium-associated, anisometric growth of the auxospore. Interestingly, a few Thalassiosirales do possess elliptical (e.g. McLaughlin, 1992) or polygonal valves (Economou-Amilli, 1979), but whether they possess a properizonium is unknown.
5. The raphid pennate diatoms (Figure 13.3 [18]) are monophyletic (Medlin et al., 2000; Kooistra et al., 2003b; Medlin and Kaczmarska, 2004). Consequently, it is most parsimonious to assume that the raphe system—a system of slits through the cell wall and specialized secretory and streaming areas of the underlying protoplast that together generate rapid surface-associated locomotion—has evolved only once. Among the raphid diatoms, it appears that the *Eunotia* group (Figure 13.1 [1]) is probably basal (Mayama and Kuriyama, 2002; Sims et al., 2006; Alverson et al., 2006), as required by the model of raphe evolution proposed by Mann (1984a).
6. Some revisions of diatom genera made using particularly extensive non-molecular data sets have received clear support from molecular data. For example, the marine genera *Lyrella* and *Petroneis* were separated from *Navicula* because of differences in valve and chloroplast type (Karayeva, 1978; Round et al., 1990; Mann and Stickle, 1993) and suggested to belong together in the same family because of apparent synapomorphies, for example, aspects of raphe structure (external central raphe endings opening into a tear-drop–shaped groove; crook-like central internal endings), chloroplasts appressed to the valves, and complex volate pore occlusions. *Rbc*L data confirm both that *Lyrella* and *Petroneis* should be separated from *Navicula*, and that it is reasonable to classify them together in the same family (Jones et al., 2005). Similar confirmation is available for other genera formerly included within *Navicula*, such as *Placoneis* (Jones et al., 2005, supporting Cox, 1987, Round et al., 1990, and Mann and Stickle, 1995) and *Sellaphora* (Behnke et al., 2004; Jones et al., 2005, supporting Mann, 1989, and Round et al., 1990).

7. Conversely, some structures that appeared similar in light and electron microscopy, and were interpreted as homologous, have been shown not to be. A good example is the fibula, defined as a bridge of silica subtending the raphe system (Figure 13.3 [19] and Figure 13.3 [20]). Fibulae are present in a dozen or more genera, which are always placed together in taxonomic treatments (e.g. Round et al., 1990), although differences in valve structure and the extent of the raphe system (whether it is only as long as the valve, running from one end to the other, or runs around most or all of both sides of the valve) lead to a grouping of the fibulate genera into three orders: the Bacillariales (Figure 13.3 [19]), Rhopalodiales, and Surirellales (Figure 13.3 [20]). In a cladistic analysis based wholly on morphological features, Ruck and Kociolek (2004) used two members of the Bacillariales (*Nitzschia scalaris* and *Simonsenia delognei*) as the outgroups for an analysis of the Surirellales. However, it has been shown by Medlin et al. (2000) that members of the Bacillariales belong to a different clade of raphid diatoms to the Surirellales and have almost certainly acquired fibulae independently. The nearest relatives of the Surirellales identified thus far are non-fibulate diatoms of the genus *Amphora* (Medlin et al., 2000; Medlin and Kaczmarska, 2004).

   Another example is the lyre-shaped area adjacent to the raphe in *Lyrella* and *Fallacia*. Originally, both of these genera were classified together in *Navicula* sect. *Lyratae* (e.g. by Hustedt, 1927–1966), and when *Lyrella* was separated from *Navicula* by Karayeva (1978) it appears that all Lyratae were to be included in the new genus. On the basis of the ultrastructure of the lyre-shaped area (plain in *Lyrella*, with an overlying porous membrane in *Fallacia*) and the characteristics of the protoplast (chloroplast number and position, division of the nucleus always on the same side of the cell rather than on alternate sides with successive divisions), Round et al. (1990) separated the former Lyratae into two genera and suggested that one, *Fallacia*, belongs close to *Sellaphora*, which lacks lyre-shaped areas. The reasons for Round et al.'s (1990) classification have never been given in detail, but *rbc*L data (Evans et al., unpublished data) show that *Fallacia* and *Sellaphora* are indeed close relatives and that neither is close to *Lyrella* and *Petroneis*.
8. Although molecular data have not yet provided all the answers about phylogeny that we might want, it is increasingly clear that morphological (cell wall) data do not on their own give robust estimates of phylogeny. There is just too much homoplasy and too few characters. The unresolved polytomies and lack of bootstrap support in morphological analyses by Edgar and Theriot (2004) and Jones et al. (2005) demonstrate this well.

SPECIES AND INFRASPECIFIC RELATIONSHIPS

Cryptic species

In a review of the species concept in diatoms, Mann (1999) noted that no truly cryptic species had been found, only species that were very difficult to tell apart by eye. Recent work on *Pseudo-nitzschia* and *Skeletonema*, e.g. by Amato et al. (2007) and Sarno et al. (2005), likewise suggests that species initially distinguished on the basis of molecular or mating data will often subsequently be found to exhibit small morphological differences: they are "pseudocryptic." However, some of the differences are so slight that the species are effectively cryptic. We have recently attempted to clarify terminology, reserving "pseudocryptic" for species that are merely difficult to identify, "semicryptic" for species that can be told apart only when the observer has both morphological data and provenance information, and "cryptic" for species that cannot be separated morphologically under any circumstances. In each case, the criteria apply to identification of individuals, not populations. Semicryptic species exhibit partial overlap in the ranges of metric characters and/or the frequencies of alternative character states in qualitative characters. Thus, for example, two genetically distinct and reproductively isolated demes currently classified together in *Pseudo-nitzschia calliantha* differ in the mean number of sectors within each valve pore, but the ranges

overlap for this character (Amato et al., 2007). In such cases, even though two species may differ significantly in the mean and dispersion of particular character states, identification of *all* individuals within a population is possible only when supplementary data are available (e.g. about variation within the whole population from which the individuals were derived, or about distributions in nature). Thus, in material from Blackford Pond, Edinburgh (which we have studied particularly intensively, e.g. Mann et al. 2004), all *Sellaphora* species can be told apart morphologically (though only with difficulty): *locally*, therefore, the *S. pupula* species complex is pseudocryptic. Elsewhere, however, genetically distinct demes can be found with morphologies that seem to overlap with the Blackford species, making purely morphology-based identification unsafe; on an international scale, therefore, variation in the *S. pupula* complex is semicryptic.

The best-studied species complexes are in the marine genera *Pseudo-nitzschia* (Orsini et al., 2004; Cerino et al., 2005; Lundholm et al., 2003, 2006; Amato et al., 2007) and *Skeletonema* (Kooistra et al., 2005; Sarno et al., 2005), and in the freshwater genus *Sellaphora* (Figure 13.3 [18]: Mann, 1989; Behnke et al., 2004). Together these studies demonstrate that assigning diatoms to individual taxa solely on the basis of morphological or physiological attributes may often be inadequate. One of the most pressing needs in micro-eukaryote taxonomy is the establishment of species definitions that are both meaningful and practical. Recently, a DNA barcoding system has been proposed, whereby all taxa would be "labelled" and subsequently identified according to the sequences of certain target genes. However, although a universal barcoding system has been supported strongly by its proponents (e.g. Hebert, et al., 2003; Blaxter, 2004), there are also many sceptics because of major unresolved issues. For example, it has not yet been determined how well molecular groupings correspond to biologically defined taxa (i.e. from morphology and breeding data), nor the range of molecular variation allowable within a barcoded unit, nor what genes are suitable in each major group. The species complexes listed above provide ideal systems in which to test rigorously the suitability of DNA barcodes in the designation and identification of diatom species (Evans et al., 2007).

In *Pseudo-nitzschia*, studies have concentrated on resolving taxonomic confusion as a result of morphological plasticity, variable toxin production, and reproductive isolation, particularly in the *P. pseudodelicatissima/P. cuspidata* (Lundholm et al., 2003), *P. delicatissima* (Orsini et al., 2004; Lundholm et al., 2006), and *P. galaxiae* (Cerino et al., 2005) species complexes. New species have been described, although insufficient sampling means that the biogeographies of these species are unknown, compared to the cosmopolitan nature of the morphospecies they replace (Hasle, 2002). Amato et al. (2007) have made a particularly detailed study of the *P. delicatissima* and *P. pseudodelicatissima* groups in the Bay of Naples and show that there is good concordance between groupings based on ITS2 gene sequences and reproductive compatibility, and that these groupings generally also show slight morphological separation. However, Coleman's (2005) study of the *Paramecium aurelia* complex shows, as might be expected, that reproductive isolation of sexual forms is not always accompanied by divergence in neutral regions of the genome. Thus, lack of significant ITS variation does not imply that speciation has not occurred.

Detailed studies have also been made of *Skeletonema* (Medlin et al., 1991; Kooistra et al., 2005; Sarno et al., 2005). As in *Pseudo-nitzschia,* morphological and molecular studies have uncovered diversity within what was previously assumed to be a single cosmopolitan species, *S. costatum*. Sarno et al. (2005) described four new species, and Kooistra et al. (2005) uncovered yet more diversity and found that the new *Skeletonema* taxa seem to be geographically confined; for example, *S. grethae* was detected only along the east coast of the United States.

In freshwater environments, intensive morphological, mating, and molecular studies have been conducted on the *Sellaphora pupula* species complex (Mann, 1984b; Mann, 1989; Mann and Droop, 1996;, Mann, 1999; Mann et al., 1999; Behnke et al., 2004; Mann et al., 2004). As in *Pseudo-nitzschia* and *Skeletonema*, there are many semicryptic and pseudocryptic species, which are morphologically similar but reproductively isolated, possess different mating systems, exhibit different degrees of genetic relationship to each other, and differ in sensitivity to parasites.

Population genetics

Population genetics is the documentation of the distribution of genetic variation within and between populations of a species and the study of the evolutionary forces (mutation, migration, selection, and drift) that structure populations genetically, i.e. produce a non-random distribution of genetic variation (Hartl and Clark, 1997). Identifying populations (collections of individuals that live within sufficiently restricted areas that any member can potentially mate with any other member; Hartl and Clark, 1997), is important to our understanding of what constitutes a diatom species, since local populations are the evolving units of a species. Having a good understanding of diatom population genetics is vital if we are to understand the dispersal and, hence, biogeography and biodiversity of diatoms.

Diatoms vary in growth-form and habitat (epipelic, epilithic, epiphytic, planktonic, etc.) and breeding systems and all of these could have important impacts on the resultant population structure. Most research to date has focused on marine diatoms and all refers to planktonic forms. No data exist for benthic species, which by their very nature may be dispersed over shorter distances and, hence, mate with neighbours more frequently, leading to more pronounced population genetic structures.

Marine

Prior to Gallagher's (1980, 1982) pioneering research into the population genetics of *Skeletonema costatum* in Narragansett Bay (Rhode Island), the levels of genetic variation present within species of phytoplankton were unknown. On the whole, authors have tended to emphasize the likely clonal nature of diatom populations, produced by the long periods of mitotic division between rare sexual events (e.g. Richardson, 1995), rather than the obligatory nature and regularity of sex in most diatoms. Using allozymes to genotype 457 isolates, Gallagher demonstrated that *S. costatum* isolates were genetically variable (Gallagher, 1980) and subsequent work illustrated an even greater degree of physiological diversity, indicating that the relatively insensitive allozyme technique failed to detect all of the genetic diversity present (Gallagher, 1982). Despite this, two separate populations were identifiable, one associated with summer blooms and the other associated with winter blooms. Gallagher (1982) suggested that these two populations could represent individuals belonging to different species and recent evidence of cryptic speciation within *S. costatum* would appear to support this (Kooistra et al., 2005; Sarno et al., 2005).

The few studies conducted over the intervening years (e.g. Skov et al., 1997) also demonstrated genetic variability within marine planktonic diatoms, but these precluded detailed analyses of population structure because of small sample size and choice of molecular marker. It was not until 2000 that the first study to use microsatellites was published (Rynearson and Armbrust, 2000). Microsatellites are repetitive regions of DNA (e.g. CA or GA units) that are found in the genomes of every organism. They are useful to population geneticists because the length of the repeat region (i.e., the number of repeats) can vary between individuals of a species and so can act as part of a fingerprint to distinguish one individual from another. They are more variable than allozymes and more reliable than RAPDs (Tingey and del Tufo, 1993), and they exhibit co-dominant inheritance (i.e., both alleles at a locus can be detected and so it can be determined whether an individual is homozygous or heterozygous at each locus), which increases the information yielded (compared to AFLPs, for example). These features make microsatellites the markers of choice for population genetic analyses. The downside is that their initial development can be very time consuming. It has been noted previously that macroalgae have fewer and less polymorphic microsatellites than higher plants (Olsen, J. et al., 2002). Although isolating suitable microsatellite loci from diatoms can also be difficult, levels of polymorphism seem to be sufficient. Rynearson and Armbrust (2004) used only three loci, but the numbers of alleles per locus ranged from 10 to 78. In the only other microsatellite-based studies published to date, Evans and colleagues isolated nine microsatellite loci for *P. multiseries* (3 to 7 alleles per locus; Evans et al., 2004) and six for its closest known

relative, *P. pungens* (6 to 24 alleles per locus; Evans et al. 2005). An additional potential problem is that diatom microsatellites appear to be more complex than those found in higher plants and animals, so that alleles can differ by just one base pair (bp) (Evans et al., 2005). Care and use of suitable positive controls are therefore required to ensure accurate genotyping of individuals, because a rise in temperature of 5˚C has been shown to affect the allele length detected during genotyping by capillary electrophoresis by up to 0.7 bp (Davison and Chiba, 2003).

Rynearson and Armbrust (2000) used microsatellite markers to investigate genetic diversity within the planktonic centric marine diatom *Ditylum brightwellii* in the inland fjord of Puget Sound (Washington). They reported high levels of clonal and genetic diversity, but the small sample size (24 isolates) limited interpretation. Subsequently, hundreds of isolates were obtained from Puget Sound and an adjacent estuary, the Strait of Juan de Fuca (Rynearson and Armbrust, 2004; Rynearson et al., 2006). Concurrently, microsatellite-based work was published on *P. multiseries* (mostly from Canadian waters) and *P. pungens* (mostly from the North Sea: Evans and Hayes, 2004; Evans et al., 2004, 2005). The *P. pungens* work (Evans et al., 2005), in particular, provides a good complement to the *D. brightwellii* studies because oceanographic conditions differ significantly between the two areas.

Both studies demonstrate a large degree of clonal and genetic variation within planktonic marine diatoms. For example, 453 of the 464 North Sea *P. pungens* isolates genotyped were genetically distinct from each other and high levels of genetic variation were maintained even during bloom periods (Rynearson and Armbrust, 2005). However, despite the similarity of the spatial and temporal scales over which the isolates were obtained (approximately 100 km and 18 months in the Evans et al. [2005] and Rynearson and Armbrust [2004] studies), the structuring of the genetic variation differed markedly between the two species. These differences are probably best accounted for by the environments from which the isolates were obtained. The German North Sea is well mixed and so the genotyped isolates probably represent one population (significant F_{ST} values between isolates belonging to different groups, classified according to time or place of isolation, were at most 0.04, indicating weak genetic differentiation). In contrast, Puget Sound and the Strait of Juan de Fuca have only limited exchange of water and *D. brightwellii* isolates sampled from these waters belonged to four different populations (significant F_{ST} values between populations were up to 0.25, indicating a high degree of genetic differentiation; Rynearson and Armbrust, 2004; Rynearson et al., 2006). Despite this differentiation, it was thought likely that all isolates were members of the same species, because 18S and 5.8S rDNA sequences of selected isolates were identical and divergence of the less-conserved ITS region was at most 1.1% (Rynearson and Armbrust, 2004; Rynearson et al., 2006). Breeding experiments between isolates from each population should now be carried out to confirm these predictions. Until appropriate species concepts are established for diatoms and other microalgae, such an integrative approach is necessary if we are to truly understand population dynamics and, hence, speciation processes.

The fact that Rynearson and colleagues detected multiple populations (one of which has persisted for at least 7 years; Rynearson et al., 2006) within a relatively small area prompts a reassessment of our ideas of speciation in the marine environment, where barriers to dispersal are often not immediately apparent and where it is generally assumed that aquatic currents disperse organisms widely. But is *D. brightwellii* atypical? Evans et al.'s (2004) findings for *P. multiseries* suggest tentatively that its populations may be similarly structured, although a small sample size was involved (25 isolates). Here, a Russian isolate introduced 11 new alleles at six loci, relative to Canadian material (Evans et al., 2004). In contrast, work on *P. pungens* (Evans et al., 2005) showed that three Canadian isolates possessed only two alleles not found among the 464 German isolates, which is surprising given the considerable geographical separation and the conflicting results for its close relative *P. multiseries*. Castelyn et al. (2004) reported that ITS sequences from North Sea *P. pungens* isolates were identical and that all clones were sexually compatible; isolates collected from further afield, including New Zealand and the Pacific coast of the United States were also sexually compatible with the North Sea isolates, with no obvious loss of viability in the F1 generation (Chepurnov et al., 2005). These results support Hasle's (2002) view that *P. pungens* is

a cosmopolitan species, which is presumably able to tolerate a wide range of environmental conditions. Before generalizations can be made, however, more studies need to be conducted, both over larger scales and in comparable environments to those from which the *D. brightwellii* isolates were obtained (Rynearson and Armbrust, 2004).

Freshwater

Work in freshwater environments has lagged behind that in the marine environment, despite the fact that it is easier to envisage potential barriers to dispersal and therefore to test hypotheses relating to the dispersal and biogeography of diatoms. Currently, little is known about how much gene flow could occur between populations of freshwater microalgae. The little information available suggests that terrestrial or subaerial algae are more easily spread than lotic or lentic species (e.g. during colonization of Surtsey: Behre and Schwabe, 1970). There are a few observations relevant to dispersal of freshwater phytoplankton (e.g. Parsons et al., 1966; Atkinson, 1971), but none that apply to freshwater benthic microalgae.

All freshwater diatom population genetics studies have focused on planktonic species and all have used molecular markers with well-known associated drawbacks (e.g. isozymes) and/or small sample sizes (*Asterionella formosa*, Soudek and Robinson, 1983, and De Bruin et al., 2004; *Stephanodiscus*, Zechman et al., 1994; and *Fragilaria capucina*, Lewis et al., 1997). The results differ but suggest overall that freshwater diatoms have limited dispersal capabilities and that geographic patterns exist, although, at least for Lewis et al. (1997), samples were collected along a broad temperature gradient and so genetic distinctiveness between populations could in part be due to thermal ecotypes.

What don't we know and what impedes us making progress?

1. Despite the addition of more taxa to molecular analyses, we do not know the branching order of the major lineages within the centric diatoms. SSU phylogenetic trees that have *Bolidomonas* as the outgroup and include several tens or >100 species representing many of the families and orders of centric and pennate diatoms show the multipolar centrics (Fox and Sorhannus, 2003; Sinninghe Damsté et al., 2004) or both the radial and the multipolar centrics (Medlin et al., 2000; Medlin and Kaczmarska, 2004, Sorhannus, 2005) as paraphyletic (see also Alverson et al., 2006; Cavalier-Smith and Chao, 2006). Even where analyses do show the radial centrics or the multipolar centrics + pennates as monophyletic, there is generally little statistical support. Thus, in a recently published SSU analysis (Sims et al., 2006, figure 1), based on thousands of sequences in the ARB database (www.arb-home.de), there is good support only for monophyly of the pennate diatoms (Bacillariophyceae), not of the radial centrics or bi/multipolar diatoms. The Bayesian analysis in Sims et al.'s (2006) figure 2 gives apparently strong support to the idea of a basal dichotomy between the radial and the [multipolar centric + pennate] clades, but this is an exception (see also Kooistra et al., 2003b; Horiguchi and Takano, 2006). Despite the paraphyly of the radial centrics and multipolar centrics in some of their analyses, Medlin and Kaczmarska (2004) decided to recognize both as classes.

   However, let us assume for the sake of argument that both the radial centrics and the multipolar centrics are indeed monophyletic. What would that mean for our understanding of diatom evolution? (1) The shape and symmetry of the ancestral diatoms would probably be unknowable, unless we discover a new sister group for extant diatoms or make remarkable new discoveries in the fossil record. This is because the bolidophytes have few or no characteristics that can be used to polarize morphological character state transitions in the diatoms. The first diverse flora of diatoms (from a Lower Cretaceous marine deposit in Antarctica; Gersonde and Harwood, 1990) includes both circular and bipolar centric diatoms (discussed by Sims et al., 2006), presumably indicating that the "radial" and "multipolar"

groups had already diverged from each other. (2) All extant multipolar centric diatoms would have to be descended from the diatom that also gave rise to all extant pennate diatoms, so that any morphological features shared by pennate diatoms and any of the multipolar diatoms would either have to be homoplasies or plesiomorphic for the whole pennate–multipolar centric clade. In addition, the origins of any autapomorphies of pennate diatoms, such as the sternum or isogamy, would probably remain unrecoverable.

2. Within each of the araphid and raphid pennate diatoms, although some groupings receive strong support, the overall course of evolution is not obvious and the origins of many groups are obscure.
3. The inability of currently used genetic sequence analyses to generate robust hypotheses about relationships and evolutionary trends implies one or more of the following: (a) the gene sequences used are not long enough (see below, point 4); (b) their evolution proceeds too quickly (producing saturation and homoplasy) or too slowly (some nodes will not be resolved); (c) taxon sampling is inadequate; (d) morphological evolution is poorly coupled to DNA sequence evolution; (e) different loci or parts of loci are giving conflicting evolutionary signals; and (f) there really were bursts of cladogenesis, perhaps during and immediately after environmental crises, e.g. at the Cretaceous–Tertiary (KT) boundary or during the less famous Triassic–Jurassic extinction event (Olsen, P. et al., 2002). Unfortunately, we do not have enough information at the moment to determine what combination of these or other factors is to blame. In relation to point (f), the fossil record does not show a massive turnover of taxa at the KT boundary (Sims et al., 2006), but it is important to remember that the fossil record is heavily biased toward planktonic diatoms, whereas the greatest diatom diversity today—and presumably therefore many key evolutionary transitions in the past—is in the benthos.
4. Most diatom phylogenies have been constructed from less than 2000 aligned nucleotides, whereas Wortley et al. (2005) suggest that approximately 10,000 may be required for "difficult" cases. Multi-gene phylogenies are surely the way forward, if we really want to know the origins of diatom diversity. Genes like *rbc*L and the more slowly evolving elements of rDNA are not unsuitable for phylogenetic analysis at most levels of the taxonomic hierarchy in diatoms; we just need more like them.

   At first sight, developing a multi-gene phylogeny looks straightforward—a simple though tedious and expensive extension to the work represented in Table 13.1. Unfortunately, it will be difficult to build on past work, because in many cases the material used for SSU rDNA or other previous studies is no longer available. This is because of the special difficulties of maintaining diatoms in culture. Many taxa have never been successfully cultivated *in vitro*—a problem that is not unique to diatoms—but even in those that can be grown, the life span of clones is generally limited by obligate size reduction (Mann and Chepurnov, 2004; Chepurnov et al., 2004). For a culture to persist, the diatom must undergo auxosporulation to restore maximum cell dimensions and allow further vegetative growth (it will then usually be genetically heterogeneous). Auxosporulation often fails, however, because the mating system may prohibit sexual reproduction within a clone (pennate diatoms are frequently heterothallic), or conditions may not permit gametogenesis, or inbred progeny may not be viable (Chepurnov et al., 2004). Thus, most of the *Sellaphora* clones used for the ITS–SSU rDNA study by Behnke et al. (2004, originally from our collections) are now dead, as are many of the clones used by Medlin and co-workers for their earlier analyses (we have searched the CCAP and CCMP collections for the strains specified). Those diatoms that survive long term in culture must lack (or bypass) a sexual cycle or must be able to tolerate inbreeding; they are the atypical weeds of the diatom world (cf. *Phaeodactylum*). Hence, making a multi-gene phylogeny of diatoms will in many cases involve starting from scratch with new cultures.

5. The uncertainties in species identification in diatoms (not only because of the existence of cryptic species but also because of fuzzy concepts of species, accidental error, or simple incompetence) mean that it is dangerous to assume that sequences recorded for a particular species do in fact represent that taxon, or that the taxon itself is a meaningful entity. For example, Sinninghe Damsté et al. (2004) used several cultures labelled *Rhizosolenia setigera*, but these appear in three places in their phylogenetic tree, on the ends of long branches. Such oddities can be studied and explained if cultures are still available. Otherwise, it ought to be possible to check the identity of the material studied, from preserved material or slides. Table 13.1 shows, however, that voucher material is rarely deposited safely in a recognized herbarium (examples where this was done are Edgar and Theriot, 2004; Behnke et al., 2004). Furthermore, to meet the highest standards of scientific reproducibility, alignments should be deposited in accessible data banks, especially alignments of rDNA (for some protein-encoding sequences, the alignment may be unambiguous). Table 13.1 shows that this ideal, too, is rarely met.
6. Many genera, families, and orders have not been sampled or are represented by one or a few species. Of the 22 orders of centric diatoms recognized by Round et al. (1990), eight remain totally unsampled, and several of the families of raphid diatoms are also missing. Furthermore, as Alverson and Theriot (2005) have noted, Round et al.'s (1990) conceptual framework was more phenetic than phylogenetic, so that their groupings may often be based on symplesiomorphies, not synapomorphies. Consequently, designing molecular sampling strategies on the basis of Round et al.'s classification will greatly underestimate the work needed to produce even a skeletal phylogeny. There is also the problem of poor coverage of taxa in culture collections, for the reasons listed earlier. However, with care, sequence data (even for two or more genes coded for in different genomes) can be recovered from a single cell whose morphology has been recorded (Sherbakova et al., 2000; Takano and Horiguchi, 2005). So, providing that we can determine in advance what set of genes is appropriate, it may not be necessary to culture "difficult" taxa.
7. Examining the relative pace and constancy of molecular and morphological evolution requires more data than we currently have, and matched data are essential. There is a considerable danger that, after molecular data have been used to group isolates into species, only one or two isolates will be studied in depth for their morphology and cytology, on the unjustifiable assumption that they are typical of their clade. Of all the studies listed in Table 13.1, only Lundholm et al. (2003) and Edgar and Theriot (2004) seem to have made a sustained attempt to obtain and analyse morphological and molecular data for the same accessions; Edgar and Theriot (2004) also included morphological data for related fossil diatoms. In a study of some raphid diatoms, Jones et al. (2005) constructed a tree from a matrix of morphological, cytological, and reproductive characters for comparison with an *rbc*L gene tree. However, the taxa included were only a small subset of those that would have been needed to examine evolution of all raphid diatoms and were selected to test only a few, very restricted hypotheses of relationships.
8. The system of families, genera, and species that we probe with molecular tools is for the most part a system that has been built up from comparative morphology of just one part of the diatom cell—the valve (for most diatomists, it almost seems as though the valve is the diatom). Mann and Cox spent several years attempting to increase the information content of classifications of raphid diatoms through studies of chloroplast morphology and sexual reproduction (e.g. Cox, 1987; Mann, 1989; Mann and Stickle, 1993, 1995), although it must be admitted that the approach taken was often as flawed as that described under point 7 above, being based on the use of "exemplar" species that it was hoped were representative of groupings based on cell wall ultrastructure. Where it has been possible to check them using molecular data, the taxonomic realignments

suggested by Cox and Mann have proved on the whole to be just (although it is also becoming evident that several new paraphyletic groups, e.g. *Nitzschia sensu stricto*, *Navicula sensu stricto*, were created as a by-product: Lundholm et al., 2002a; Simpson and Mann, unpublished). By contrast, genera based wholly on wall characters—whether the genera are long established or recently described—often prove to be poly- or paraphyletic. Examples are the raphid genus *Eolimna* (Schiller and Lange-Bertalot, 1997), which is polyphyletic (Beszteri et al., 2001; Behnke et al., 2004), and the centric *Thalassiosira*, which is apparently paraphyletic (Kaczmarska et al., 2006), despite being revised rigorously by Hasle and others using SEM data (producing the system summarized by Hasle and Syvertsen, 1996).

9. However, the principal deficiency is that, even when robust phylogenies are available, they are often profoundly unsatisfying. The information content of the existing classification is low, having been built almost exclusively from valve data (although we acknowledge that it is nevertheless richer than in many other protists). Hence, molecular phylogenies of diatoms easily become little more than exercises in linking names together. Sometimes there have been attempts to plot information about morphological or cytological characters onto gene trees, but this is generally done "by eye" (e.g. Medlin and Kaczmarska, 2004), rather than by formal reconciliation using some specified criterion such as parsimony, and the information is often so sparse and inconsistent as to make generalization dangerous: Sims et al. (2006) are notably more cautious about correlations between molecular phylogenies and non-molecular data, compared to Medlin et al. (2000).

   What is lacking is a science of "diatomics": a concerted, consistent attempt to survey, record, and categorize the morphological, cytological, nuclear, growth, reproductive, and other characteristics of diatom species. Gathering such information can be easy (plastid morphology, gross nuclear structure) or difficult (determining protoplast ultrastructure from thin sections) or time consuming (examining auxospore formation and development), but without it, molecular phylogenies will often be trees of name tags.

10. We turn now to infraspecific variation. We are just beginning to understand gene flow between microbes in marine environments, and it is becoming increasingly apparent that population dynamics are much more complex than had been assumed. Barriers to gene flow exist in seemingly open aquatic environments, as do locally adapted populations, which helps to explain how diversity in marine systems has arisen and why it is higher than has been concluded from surveys based on morphospecies concepts. Now that microsatellite markers exist for a few species, progress in our understanding of phytoplankton population dynamics should be much more rapid. *Pseudo-nitzschia pungens* is an apparently cosmopolitan species and has been the focus of detailed studies of its life history (Chepurnov et al., 2005); it therefore serves as an ideal model. Understanding the bloom dynamics of its closest relative, *P. multiseries,* a diatom often connected with outbreaks of amnesic shellfish poisoning, should be a priority in view of the differences that seem to exist in genetic structure between *P. multiseries* and *P. pungens*. There is some evidence for cross-amplifiability of microsatellites between these two species (Evans and Hayes, 2004; Evans et al., 2004). The increasing amount of information available from the *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* sequencing projects (projects to sequence the *Fragilariopsis cylindrus* and *Pseudo-nitzschia multiseries* genomes are in progress: information on these is available at www.jgi.doe.gov/sequencing/index.html) will aid the development of markers (both neutral and those potentially under selection) in these species and their closest relatives, though the absence of any good information about the distribution and ecology of *Phaeodactylum* will be a severe hindrance to interpretation. Studies that assess population differentiation and gene flow need to be carried out on a global scale to determine the extent of human-mediated dispersal (e.g. due to transportation of cells in ships' ballast water) versus dispersal via oceanic currents.

11. No explanation of diatom evolution can be adequate if it does not address the diversification of benthic diatoms, which outnumber planktonic species by an order of magnitude or more. No population genetic studies have yet been made of benthic diatoms, and we have therefore begun work on the population genetics and biogeography of the *Sellaphora pupula* species complex. This work should also allow the first detailed comparisons between marine and freshwater: does the division of the habitat into lakes or rivers ("water islands and isthmuses") constitute more effective barriers to gene flow than are ever present in the sea?
12. Other questions that need to be answered to aid our understanding of population dynamics include the fate of cells between bloom periods, especially for diatoms that (apparently) lack resting stages. Also, methods to measure the incidence of sexual reproduction in field populations need to be developed, because direct observation is impractical and it is therefore hard to assess the importance of sex in the production and maintenance of the high levels of genetic diversity that have been detected (Evans et al., 2005). In North Sea *P. pungens* populations, sexual reproduction appears to occur frequently because all six microsatellite loci were in Hardy–Weinberg equilibrium (Maynard Smith, 1989). It is only through conducting such research that a true understanding of the population genetics, dispersal, biogeography, and biodiversity of microalgae will be gained. Until then, the general (and possibly highly misleading) consensus (cf. Finlay and Fenchel, 2004) will continue to grow, that microorganisms do not possess biogeographies and are relatively species poor.

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