

Morphogenetic information and the selection of taxonomic characters for raphid diatom systematics

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Background – The traditional approach to diatom systematics assumed that structure and morphology were stable within a taxon and replicated faithfully down the generations, but wall structure is the end-product of biological processes, potentially modified by environment and over time. Electron microscopy revealed additional structural characters that may be taxonomically informative, but homology continues to be inferred primarily on morphological similarity, without reference to ontogeny.

Review – Morphogenetic data reveal that similar structures can be derived in different ways, and that dissimilar end-products may be produced by a single ontogenetic pathway. This paper discusses the importance of understanding valve morphogenesis before wall characters and character states are chosen and described, with particular reference to dorsiventrality, solid areas of silica, raphe and pore structure.

Conclusions – A better understanding of character formation will inform the use and development of terminology, which is critical to comparative studies and phylogenetic analyses of morphological data.

Key words – character choice, character state, homology, morphogenesis, raphid diatoms, systematics, terminology.

INTRODUCTION

Although the development of molecular phylogenetic tools is challenging some traditional ideas, diatom systematics remains very much the product of 19th and 20th century light microscopical investigations of their siliceous cell walls. Diatom systematics has been strongly influenced by a classification that aimed to aid identification (Smith 1872), placing emphasis on shape and symmetry. Cleve (1894) on the other hand considered that such features could be susceptible to variation and were useful only at the specific, rather than generic, level. Cleve's (1894) speculations on the interrelationships of naviculoid genera reflect his attempt to develop a natural system, although he resorted to an artificial key at the start of his synopsis to help the reader in identification. This contrast perhaps also epitomises two approaches to studying diatom frustules, the one (traditional) that emphasises the stability of structure and morphology and their reliable replication between generations, and the other that sees structure and morphology as the product of biological processes that may be affected by time and environment. The traditional approach inevitably focuses on the end-product, the mature valves and cingulum, whereas a more biological or evolutionary approach recognises that morphology and structure can be transformed. However, an awareness of the possibility of morphological variation and structural transformation raises its own challenges; what constitutes a particular char-

acter, and how might different expressions of that character be described? Which characters are informative and how do we define them?

This paper will consider how the choice of taxonomic characters for diatoms has changed with the development of electron microscopy and morphogenetic studies. It will also discuss how we should define characters (and their states) in the light of ontogenetic evidence, pointing out some of the implications for diatom systematics, with particular reference to raphid diatoms.

CHARACTER CHOICE AND DEFINITION

As mentioned above, by the end of the 19th century light microscopical studies of diatoms focused on features of their siliceous frustules, especially the shape, symmetry and stria pattern of valves, and three main groups came to be recognised, centric, araphid pennate and raphid pennate diatoms (Schütt 1896). This focus was maintained through the first half of the 20th century, but with the development of electron microscopy, especially scanning electron microscopy, the diversity of wall ultrastructure was revealed (see survey of morphology in Round et al. 1990), expanding the range of characters available to the diatom taxonomist (Cox 2003a, in press). Thus the traditional characters of shape, symmetry, raphe system, stria arrangement, presence and type of

processes, presence or absence of pore fields and/or isolated pores, shape and extent of central and other hyaline areas, as well as the presence or absence of longitudinal canals or other less common features were augmented by more structural information. Variation in raphe construction was revealed, particularly of the raphe slits and associated ribs, different types of pores and their occlusions, types of processes, pore fields and isolated pore structure. In addition, it became clear that, while some parts of valves of particular taxa were solid from their inception, in other taxa solid areas developed by siliceous infilling later in development (Cox 1999b, 2001).

The incorporation of ultrastructural information into taxonomic descriptions has resulted in the description of many new genera, e.g. *Biremis* D.G.Mann & E.J.Cox, *Decussata* (Patrick) Lange-Bertalot, *Fallacia* Stickle & D.G.Mann, *Fistulifera* Lange-Bertalot, *Lyrella* Karayeva, *Parlibellus* E.J.Cox, *Petroneis* Stickle & D.G.Mann, *Psammodictyon* D.G.Mann, *Reimeria* Kociolek & Stoermer, *Seminavis* D.G.Mann (Round et al. 1990, Lange-Bertalot 2000, Lange-Bertalot 1997), and the resurrection of several “old” genera, e.g. *Berkeleya* Greville, *Diadesmis* Kützing, *Muelleria* Frenguelli, *Placoneis* Mereschkowsky, *Sellaphora* Kützing (Round et al. 1990, Spaulding & Stoermer 1997).

In many cases, the inclusion of diverse groups within pre-existing genera was suspected and had been reflected in the creation of infrageneric taxa within those genera (Hustedt 1959–1966, Patrick 1959a, 1959b). Thus, many new raphid genera have been created for former sections or subgenera (or parts thereof) of *Navicula* Bory (e.g. *Decussata*, *Haslea* Simonsen, *Lyrella*, *Muelleria*, *Parlibellus*, *Proschkinia* Karayeva, *Petroneis*) and the description of new genera of raphid diatoms continues (Johansen & Sray 1998, Vyverman et al. 1998, Maidana & Round 1999, Metzeltin & Kusber 2001, Lange-Bertalot et al. 2003, Levkov et al. 2007, Gligora et al. 2009). However, it is when comparisons are being made between the type species of existing genera and representatives of potentially new genera that the choice and the description of distinguishing characters become particularly important (Cox 2002b, 2003b). If only the features of the generitype are considered, rather than the entire spectrum of character states within the genus, new, narrowly defined, numerically small, genera are more likely to be created. It is also important that comparisons are made with a range of other genera, not just those in similar habitats, and that real efforts are made to evaluate the phylogenetic relationships of putative new genera, and to integrate them into the broader systematic arrangement of diatoms (Cox 2009).

In the majority of instances, observations are made on cleaned field material, which contains a variety of taxa, usually represented predominantly by fully mature valves. That only mature valves are recorded may be both a reflection of the timing of cell division and new wall formation (usually during the dark period for raphid taxa) (Cox 1981, Gillard et al. 2008, Cox unpubl. res.) versus the timing of field collections (during the day), and the natural tendency of observers to select “normal-looking” valves, rather than those with poorly consolidated surfaces or those that are overtly incomplete. However, the natural occurrence of significant numbers of forming valves can be used serendipitously to study the process of wall formation (Tiffany 2002). Obtaining compre-

hensive morphogenetic information on most diatoms requires at least some culturing work (Cox 1999b, 2001, 2006), although this may be less demanding than many think.

MORPHOLOGY AND THE DIATOM LIFE-CYCLE

Because, after mitosis, new valves are formed within the confines of the parent frustule, most diatom populations show gradual size diminution as they multiply (MacDonald 1869, Pfitzer 1869). However, size reduction is allometric not proportional (Cox 1993), the long axis of pennate diatoms usually decreasing significantly, while valve width shows little change (and may even occasionally increase slightly). Thus, valve outline invariably changes, shorter valves appearing “fatter” than longer ones and many shapes become more rounded, but apical outline does not invariably change with size and occasionally valve undulations can become more marked (Hustedt 1955, Cox 1986). Heteropolar diatoms, such as *Gomphonema* Ehrenberg, often show shape change at their head pole, whereas the foot pole is unchanged (Dawn Rose unpubl. res.), presumably because of the functional role of the apical pore field (foot pole) in mucilage secretion and attachment. Similarly, there is little reduction in raphe slit length in *Berkeleya rutilans* (Trentepohl) Grunow with size reduction, but the “non-functional” central area becomes shorter (Cox unpubl. res.). On the other hand, stria and pore densities vary little with changing valve length (although stria density may change due to environment), and other structural components, e.g. pore size and structure, are unaffected by size reduction (Cox 1983).

Restitution of maximum size occurs via auxospore formation (usually, but not invariably, the result of sexual reproduction), which then expands outside the constraints of the frustule but within a perizonium, and subsequently forms the initial cell, whose division restarts the vegetative cycle. The first formed initial valves show most of the structural features of vegetative valves, but tend to differ in shape and valve curvature, while their patterning is often somewhat irregular (Mann 1982, 1989, Cohn et al. 1989, Passy-Tolar & Lowe 1995, Sato et al. 2004, 2008). Only after mitosis of the initial cell are ‘typical’ vegetative valves formed (e.g. Sato et al. 2008). Most frequently, initial valves differ from vegetative cells in stria spacing, although pore spacing along the striae does not (Cohn et al. 1989). It has been suggested (Schmid 1994) that the ‘errors’ in initial valve formation reflect a delay in reconstituting the cell’s cytoskeleton, which modulates valve morphogenesis, while Passy-Tolar & Lowe (1995) suggest that the initial cell may reveal more primitive character states.

ESTABLISHING CHARACTER HOMOLOGY

All comparative morphological studies rely on some inferences of character homology, i.e. that similar structures are a result of evolutionary differentiation from a common ancestor. Without such inferences, comparisons are impossible. That this has been assumed for diatoms is reflected by the use of the same term for similar features across taxa. But morphological similarity may be given undue significance when features in different parts of a frustule are compared.

Thus, rimoportula is applied to all processes with a lipped internal opening and a simple pore-like external opening, regardless of position on the valve, i.e. marginal or more or less central, or association with other features, e.g. with pore fields in some araphid diatoms, or a rudimentary raphe slit in the Eunotiales. Similarly, within the raphid diatoms the term stigma has been applied to one or more isolated pores near central raphe endings, yet ultrastructural investigations have revealed that such isolated pores exhibit a variety of internal occlusions and their homology should perhaps be questioned. In this case, use of the single term, stigma, is probably a result of its coinage for LM study, but the appropriateness of the term has not been re-evaluated using SEM data (Cox in prep.).

Homology of raphe slits across the Bacillariophycidae is supported by their ontogeny and function, but it is still unclear whether the short slits of the Eunotiophycidae (sensu Round et al. 1990), which do not form the pattern centres of those diatoms, and are often accompanied by a rimoportula, the presumed precursor of the true raphe slit, are homologous with the true raphe (Cox & Kennaway 2004). Elsewhere, bilaterally asymmetrical diatoms are often described as dorsiventral, the dorsal margin having greater convexity than the ventral, but in some taxa the 'dorsal' side is the primary side of the valve, whereas in others it is the secondary side. In other words, dorsal (or ventral) margins are not invariably ontogenetically comparable.

Another problem area is that of stria and pore descriptions. Based on light microscopy, any row of pores (or presumed row of pores) was referred to as a stria. The pores within the striae were first referred to as puncta (because they appeared like dots), but are now generally referred to as areolae (poroid or loculate depending on their ultrastructure) with a variety of terms having been coined to describe different types of occlusion (Ross et al. 1979, Mann 1981a, Cox 2004). However, how the pores within striae are defined during morphogenesis varies between taxa, and it may be pertinent to ask whether all areolae are homologous.

The opposite problem arises when homologous structures no longer closely resemble each other. The tendency then is to use different terms to describe them, which may lead to different phylogenetic inferences. Until it was learnt that monoraphid diatoms infill the raphe system on one valve (Andrews 1981), it was assumed that they were intermediate between araphid and biraphid diatoms. Thus, the evolutionary sequence was presumed to be: no raphe – raphe on one valve – raphe on both valves. Monoraphid diatoms are now known to be derived from biraphid taxa (Boyle et al. 1984, Mayama & Kobayasi 1989), so the evolutionary sequence is: no raphe – raphe on both valves – raphe on one valve. Elsewhere, the proper interpretation of the peripheral raphe system of the Surirellales D.G.Mann rests on morphogenetic data that showed that one of the nodules between the raphe slit endings corresponds to the central nodule, while the other corresponds to two fused polar nodules (Pickett-Heaps et al. 1988).

CHARACTERS IN NEED OF RE-EVALUATION

Given some of the incorrect assumptions about the homology

of diatom features, a number of characters of raphid diatoms require re-evaluation. These include descriptions of features in relation to parts of the valve, and of some features themselves, a few of which are discussed here.

Dorsiventrality

As mentioned above, valves are described as dorsiventral when their margins show unequal curvature, the more convex being termed dorsal, the less convex (straight or concave) ventral. Thus *Cymbella* s. lat. included variously shaped valves, with a variety of valvar features, but also contrasting chloroplast configurations. The opposite deflection of the polar raphe endings within the genus had long been known, but its significance had not been realised (Mann 1981b). Two types of dorsiventrality exist within *Cymbella* s. lat.: one in which the primary side of the valve is more strongly curved (e.g. *Encyonema* Kützing), the other in which the secondary side is more curved (e.g. *Cymbella* C.Agardh). Correspondingly, the centre of the chloroplast lies adjacent to the ventral or the dorsal side of the cell, while the nucleus lies against the dorsal or ventral respectively; the nucleus always lying under the primary side of the cell (Mann 1983). There are also other valve features that are correlated with this distinction (Cox 2002a), all indicating that the Cymbellaceae requires re-evaluation.

A similar tendency to describe raphe fissure curvature in relation to the sides of the valve creates problems within the Nitzschiaceae. Because the raphe system is often strongly lateral and keeled, one side of the valve is often described as the valve face, the other (narrower) the valve mantle. As with other raphid diatoms, curvature of the polar raphe fissures is usually towards the secondary side of the valve, but because the incipient raphe systems move laterally during morphogenesis (Pickett-Heaps 1983), the primary side of one sibling valve will be wide (= valve face), whereas it will be narrow (= valve mantle) in the other. The raphe fissures will curve towards the secondary sides, i.e. the mantle in one, and the valve face in the other. Direction of polar raphe curvature in relation to the valve 'face' or 'mantle' is therefore meaningless.

Hyaline or thickened areas

Particular types of hyaline or thickened areas on their valves have been used as distinguishing features for some raphid genera, e.g. the presence of a transverse stauros for *Stauroneis* Ehrenberg, lyrate areas for *Lyrella* and *Fallacia*, longitudinal canals in *Neidium* Pfitzer, while variation in extent and shape of hyaline areas help discriminate species in some genera, e.g. *Pinnularia* Ehrenberg. Two terms have been used for transverse areas, a stauros and a fascia, which Cleve (1894) attempted to distinguish, considering the first an expansion of the central nodule (usually thickened), the second an expansion of the central area (Cox 2001). However, without morphogenetic information, or assuming that a stauros is invariably thicker than the virgae, it is rather difficult to distinguish between these. Yet, in theory, any transverse hyaline area can be formed in one of two ways, as a solid expanse of silica or by infilling between virgae. Morphogenetic studies reveal that the former occurs in *Stauroneis* and *Craspedos-*

tauros E.J.Cox, whereas in *Pinnularia* there is later infilling between virgae (Cox 2001). Thus, Cleve (1894) was correct in seeking to distinguish these two features, but discrimination in mature valves may remain difficult.

Longitudinal canals and hyaline or thickened areas

In a similar manner it is possible that longitudinal canals, hyaline and thickened areas could develop in two different ways. Either stronger longitudinal connections between virgae (at a distance from the raphe sternum) develop before the vimines start to form and define areolae, which may allow two divergent wall layers to form, or a longitudinal row of vimines are thickened subsequently, which would allow longitudinal hyaline or thickened areas to be formed. The former phenomenon is known to occur in *Luticola* and *Diadesmis* (cf. Cox 2006), which supports a close relationship between these taxa, and there is evidence that it is also associated with the development of longitudinal canals in *Neidium* Pfitzer (Paul Hamilton, Canadian Museum of Nature, pers. comm.) and *Diploneis* Ehrenberg (Cox in prep.). The continuation of the lines of the striae across the lyrae in some *Lyrella* spp. may indicate that the lyrae are formed after pores have been defined, but this requires further investigation.

Pore occlusions

With the exception of pores comprising apical pore fields, which appear to be simple holes in the wall, pores through diatom valves are invariably occluded in some way, at one or other, or both surfaces of the valve (Mann 1981a, Cox 2004). The types and combinations of occlusion are taxonomically informative, but their description and interpretation is not necessarily consistent across groups (Cox 2004, 2009). Unusually two types of occlusion have been found in a single taxon (Mayama & Fujita 1995), which raises interesting questions about the controls on occlusion development. Transitions between forms of occlusion have rarely been considered, however, it is clear from observations of developing valves, that morphologically distinct external pore occlusions may be parts of the same morphogenetic pathway. Thus the variation in degree of openings between *Haslea*, *Gyrosigma* Hassall, *Navicula* s. str. and '*Hippodonta*' Lange-Bertalot, Metzeltin & Witkowski is part of a single morphogenetic pathway (Cox 2002a: fig. 24.9, in prep.). Another example is seen in the external pore openings of members of the Cymbellales (sensu Round et al 1990). These vary in their shape and orientation, from linear to curved, undulate to cruciform, and may be slightly sunken because there is additional external thickening, or even small spinules around them (as in *Didymosphenia* M.Schmidt). However, in most cases the external occlusions are thin flap-like outgrowths, different degrees and orientation of outgrowths accounting for the morphological variation (Cox 2004). The different shapes and orientation of the pore openings should therefore not be regarded as different characters, but as variants on a particular type of occlusion.

ONTOGENY AS A GUIDE TO CHARACTER TRANSFORMATION

If the developmental pathways producing particular structures are known, it is possible to identify those that follow the same morphogenetic trajectory, and those that, despite producing similar final structures, do not. This allows characters to be discriminated on their ontogenies, rather than simply their final appearance, and can also help in the recognition of character states. It may still be difficult to define where one state ends and another begins, but this would probably have less impact on a phylogenetic analysis than treating ontogenetically distinct characters as one, or transformations of a single character as different characters.

Internal thickenings beside raphe slits

Within the Naviculineae, the internal raphe sternum is often flanked by one or more accessory and/or central ribs (Cox 1999a, 2002a, b) that vary in extent and degree of thickening between species and genera. In the Naviculaceae (including *Navicula* s. str., *Haslea*, *Trachyneis* Cleve) a rib occurs on one side of the sternum only, although this may be interrupted at the centre (Cox 1999a: figs 61, 63–74), whereas in the Pleurosigmataceae sensu Round et al. (1990), two short ribs are usually present on either side of the central raphe endings. The former would be described as an accessory rib, the latter as central ribs, and they are initiated in different parts of the valve. However, some species of *Gyrosigma* have a short rib on one side of the central raphe endings, and an extended one on the other.

Accessory rib ontogeny shows that the continuous rib in *Navicula* and *Haslea* commences as two, one beside each raphe slit, and that these subsequently fuse at the centre of the valve (Cox 1999b: figs 38, 41). It would therefore be more correct to describe *Navicula* as having two accessory ribs that may fuse at the centre of the valve to form one continuous structure. Central rib ontogeny begins at the centre of the valve, extending and tapering towards the poles. In *Gyrosigma*, both central and accessory ribs can be present, fusion often occurring between the accessory ribs and central rib on the primary side of the valve (Thaler & Kaczmarek 2009: figs 6, 10). Other variations of the accessory ribs include the development into short, more or less terminal flanges in *Haslea* (Cox 1999b: figs 39, 40) and longer more central flanges in *Trachyneis* (Round et al. 1990: 569), both curving over the internal raphe sternum.

External pore openings

As mentioned above, the variation in external pore openings between some of the Naviculineae is a function of their ontogeny. In *Haslea*, the initial framework of pores formed by the virgae and vimines is overlaid by external longitudinal strips of silica, so that the external slits of pores in adjacent striae form continuous lines (Cox 1999b: figs 43–46). Where there is partial infilling between the strips above the virgae, as in *Gyrosigma littorale* (W.Smith) Cleve, two or three pores in adjacent striae share the same external slit (Cox 1979: fig. 11), and in some *Navicula* spp., pores appear almost confluent, with grooves above the virgae (Cox 1999a: figs 24, 38,

41, 42). The transition from single linear slits (in most *Navicula* spp.) to paired external openings, as seen particularly in *Navicula* ('*Hippodonta*') *capitata* Ehrenberg but also intermittently in *Navicula reinhardtii* Grunow (Cox 1999a: figs 44, 45, 47, 48), is the result of forming an additional cross-connection. However, such double pores should not be confused with the biseriate arrangement in some *Gomphonema*, e.g. *G. acuminatum* Ehrenberg, where two rows of pores are formed by offset vimines development (Cox 1999b: 64–68).

Alveolate striae

Cox & Ross (1981) re-evaluated some of the terminology used for striae in pennate diatoms, and also suggested that the term alveolus be replaced by alveolate striae, reflecting its development from a row of areolae. This is borne out by morphogenetic observations on *Pinnularia* (Cox 1999b: figs 85–99), which reveal that internal flanges grow inwards to partially occlude the striae. In other words they are modified striae, not a different structure as might be implied by the use of alveolus as opposed to stria to describe them (Cox & Ross 1981). The internal laminae of *Gomphoneis* Cleve, which also grow inwards to partially occlude its striae are homologous to the flanges forming the alveolate striae of *Pinnularia*. Thus both genera should be described as having alveolate striae, although it may be necessary to discriminate those in which the occluding flanges are primarily axial, primarily marginal, or more or less equal.

DISCUSSION

Diatom valve (and frustule) structure is the result of dynamic cellular processes over a period of time, reflecting the cell's responses to several, variously interacting factors, both intrinsic and extrinsic. In interpreting wall structure, it is therefore important to consider the morphogenetic pathways that produce different morphological characters, and to be aware of the ease, or otherwise, with which morphology can be transformed. (Teratological forms may also be of relevance here by revealing how extreme environments can modify or disrupt valve morphology.) It is clear that some structural characters are highly consistent within raphid taxa, e.g. raphe construction, type of pore occlusion (cribrum, hymenes, etc.), but that others, e.g. degree of surface silicification, final pore shape, raphe fissure path may be more susceptible to variation. To what extent these are a function of the external environment requires further investigation, but just as particular cell shapes or colonies may be more successful in certain habitats than others, so shifts in morphology may play a role in cell survival, or simply reveal that there is no single 'ideal' morphology for any one taxon in any one habitat.

Descriptive terminology is a form of shorthand, but one that must be applied accurately and consistently if it is to be informative. Thus, the creation of duplicate terms for the same structure is as misleading as using a single term for two (or more) different structures. Shared terminology inevitably carries an inference of homology. While it may be understandable that historical terms derived from LM were necessarily best approximations, with the development of EM it behoves us to be more precise and circumspect in our

use of descriptive terminology. Furthermore, understanding how particular structures are formed should inform our use and development of descriptive terms. The way in which we describe an object affects how we compare it to other objects and then classify it. Understanding the ontogeny of wall characters is an aid to their discrimination and description. As an aid to the recognition of homology, it can also contribute to phylogenetic studies based on morphology, and if translated into systematic arrangements, improve the phylogenetic content of the latter.

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