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Fourier shape analysis and fuzzy measure shape group differentiation of Great Lakes *Asterionella* Hassall (Heterokontophyta, Bacillariophyceae)

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Abstract

Species separation of character-poor *Asterionella*, an abundant Great Lakes diatom, is difficult to accomplish by visual inspection alone. Diatom shape is an inherited property which is measurable. Quantifiable variation is mostly the result of genetic influences, while qualitative changes in valve morphology are mostly the result of environmental influences. *Asterionella* valve shape and shape group separation was studied using principal components analysis (PCA) and fuzzy measure theory.

To quantify shape, Fourier coefficients were calculated from arc lengths and tangent angles around the periphery of the valve outline. Polar Fourier coefficients resulted from an orthogonal polynomial regression of $\Phi^*(t) = a_0 + \sum_n^N A_n \cos(nt + \alpha_n)$, where a_0 is the zeroth Fourier coefficient, A_n is the nth amplitude, and α_n is the nth phase angle. One-hundred x,y coordinates were used to calculate 22 shape coefficients to get a best fit closed curve in a least squares sense.

Standardized PCA of mean-corrected, square root transformed amplitudes produced seven *Asterionella* shape groups. Classification integration and fuzzy measures were used to determine degree of shape group overlap and degree that specimens belonged to an assigned shape group.

Fuzzy measures were based on morphometry of head pole, foot pole, and mid-valve widths or a combination and scaled and ordered on the interval [0,1]. Sugeno's or the fuzzy integral, $E = \int h(x) \circ g(\bullet)$, where E is the evaluation of $h(x)$ (partial evidence) and $g(\bullet)$ (importance or possibility measure), was used. Partial evidence was fuzzy average overlap. Degree of shape group membership was evaluated as degree of certainty (partial evidence) and Sugeno's measure (importance measure). Complete overlap or specimen inclusion was equal to one, complete lack of overlap or specimen exclusion was equal to zero, and the crossover point was 0.5.

Two exceptions, shape groups II-III and shape groups IV-V at $E = 0.6$ exhibited overlap. Two specimen assignments were slightly questionable at $E = 0.49$ and $E = 0.57$ for shape groups II and VI, respectively. All other specimen assignments were $E \geq 0.6$. Overall, shape group differences were evident and may indicate different species of *Asterionella* Hassal.

Introduction

Asterionella Hassall is one of the more character-poor diatoms. This genus has received little attention (Körner 1969) compared to other diatom genera have with

respect to species differentiation. Yet, it is one of the better recognized diatoms from the Great Lakes microflora because of its shape.

To evaluate valve shape as a morphological character, consideration of life cycle changes and valve construction are necessary. Shape changes with respect to size are interdependent and crucial in species differentiation and classifications (Williams 1990). Shape is a measurable quantity which can be determined by orthogonal polynomial regression (Zahn & Roskies 1972). For diatoms in general, genetics is more influential in quantitatively definable phenotypic variation than are environmental influences (Pappas & Stoermer 1996).

Diatom shape is a directly inherited property. Shape of the mother cell determines the shape of daughter cells (Schmid 1994, Mann 1994). From the plasmalemma, the cell wall as well as new valves and girdle bands form (Stoermer *et al.* 1965, Schmid 1987). Outline of one daughter cell conforms to the internal form of the parent cell while the other daughter cell is slightly smaller.

During cell division in the silica deposition vesicle (SDV), valves specific to a particular species are formed (Stoermer *et al.* 1965, Schmid 1994). In pennate diatoms, a membranous tube running the entire length of the future valve expands laterally. That is, the initiation site of SDV formation is an axial rib. SDV is next to the plasmalemma created during cleavage. The size of siliceous bands, hoops, or plates of the perizonium controls the characteristic elongate shape of pennates (Schmid 1994, Mann 1994, Edlund & Stoermer 1997).

The plasmalemma is involved in effecting turgor change and serves as a molding surface for the siliceous part of the valve (Schmid 1987, 1994). From turgor pressure, shape is maintained in pennates internally by the cytoskeleton and externallly by the cell wall (Schmid 1987, 1994; Mann 1999). Turgor pressure changes as vegetative reproduction occurs. The molding surface for new valve topography is the cleavage furrow. The cleavage furrow is shaped by turgor, local contractions, and tension of the new membrane and cytoplasm, while adhered to the cell wall. The cleavage furrow is important in adhesion of daughter to mother cells. The last girdle band deposited bears structures which enhance adhesion and prevent the two thecae from sliding apart during cell division. Shape of the cleavage surface determines shape of future valves and depends on turgor (Schmid 1987, 1994).

Relating valve shape change to life cycle stage would be highly useful in diatom taxonomy. Shape loss during size reduction and the potential for misidentification of diatoms is evident. There is a need for quantitative description of valve shape to augment studies of diatom reproduction and morphology. Coefficients from orthogonal polynomial regression can be used to represent life cycle changes for one morphological character, namely, valve shape.

In previous quantitative diatom shape studies, multivariate statistical analyses have been used in shape group separation for *Gomphoneis* Cleve (Stoermer & Ladewski 1982, Stoermer *et al.* 1984), *Tabellaria* Ehrenberg (Theriot & Ladewski 1986, Mou & Stoermer 1990), *Didymosphenia* Schmidt (Stoermer *et al.* 1986), *Eunotia* Ehrenberg (Steinman & Ladewski 1987), and *Surirella* Turpin (Goldman *et al.* 1990). In our

study, quantitative shape analysis was applied to *Asterionella* specimens from the Great Lakes. Shape group separation included using the multivariate statistical method principal components analysis (PCA) and application of fuzzy measure theory for testing shape group specimens with respect to life cycle stage.

Methods

ASTERIONELLA SPECIMENS

All *Asterionella* specimens chosen for analysis were whole and apparently flat and in an unobstructed valve view. After initial inspection of over 300 specimens, 96 qualified for use in this study. They were from the permanent slide collection of E. F. Stoermer, Center for Great Lakes and Aquatic Sciences, University of Michigan, Ann Arbor, MI 48109-1090. All Great Lakes samples were obtained with vertical net tows with a #20 plankton net (Stoermer & Yang 1969), and slides from these samples were mounted in Hyrax. Specimens available for analysis were selected from Lake Michigan, Lake Huron and Lake Superior slides.

A Leica DMRX microscope equipped with a PL APO 100x, 1.40 oil immersion objective and immersible condenser was used for viewing specimens. Digital imaging was accomplished with a SONY 3CCD camera model 960MD, and subsequently a DKC Sony 5000 camera and NIH imaging software (version 1.62) written by Wayne Rasband at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/. In addition, length, mid-valve width, head pole width, and foot pole width were measured in NIH imaging software. For more details on obtaining measurements see Stoermer (1996).

SHAPE ANALYSIS

The method of arc lengths and tangent angles was used to calculate polar Fourier coefficients from the shape function, $\Phi^*(t)$ (Zahn & Roskies 1972, Bennett & Mac Donald 1975, Persoon & Fu 1977), which is

$$\Phi^*(t) = a_0 + \sum_n^N A_n \cos(nt + \alpha_n),$$

where n through N Fourier coefficents are calculated, and the mean coefficient, a_0, is

$$a_0 = -\pi - \frac{1}{L}\sum_{k=1}^{M} l_k \Delta\phi_k$$

for the kth arc length, l, and kth change in angular bend, ϕ, for M x,y coordinates, and total arc length, L. Amplitude is

$$A_n = \sqrt{a_n^2 + b_n^2}$$

and phase angle is

$$\alpha_n = \arctan\frac{b_n}{a_n}$$

from Fourier coefficients

$$a_n = -\frac{1}{n\pi}\sum_{k=1}^{M} \Delta\phi_k \sin\frac{2\pi n l_k}{L}$$

and

$$b_n = \frac{1}{n\pi}\sum_{k=1}^{M} \Delta\phi_k \cos\frac{2\pi n l_k}{L}.$$

To preserve pseudolandmarks, 22 Fourier coefficients were calculated from 100 x,y equidistant-spaced coordinates for each specimen. Valve shape outline was reconstructed for each specimen to ensure that Fourier coefficients represented a best-fit closed curve in a least squares sense.

PRINCIPAL COMPONENT ANALYSIS

To determine shape variation across all specimens, PCA from the statistical package CANOCO™ (Ter Braak 1988) was used to ordinate amplitudes from each specimen divided by the mean or zeroth coefficient, a_0 (Younker & Ehrlich 1977).

Data transformation was used on mean-corrected amplitudes to improve symmetry about the grand mean (Noy-Meir 1973, Noy-Meir *et al.* 1975). Centering and standardization (mean equals zero, variance equals one) was used to rescale the data centroid to the origin as well as rescale the variance around the origin (Noy-Meir *et al.* 1975, Jongman *et al.* 1987, Ter Braak 1988). That is, contribution of each specimen was proportional to its variance (Noy-Meir *et al.* 1975), and equal contribution of all specimens means that any given specimen would not unduly influence the ordination. In addition, to further disperse the data around the grand mean, square root transformation was used.

Standardized unit variance component scores (loadings) were determined to produce correlation between specimen arrows (Cooley & Lohnes 1971, Green & Carroll 1978, Jongman *et al.* 1987, Ter Braak 1988). Since standardization was used, only specimen scores were ordinated rather than a biplot including scores for each harmonic (Noy-Meir *et al.* 1975, Gower & Hand 1996).

FUZZY MEASURE THEORY

From fuzzy measure theory, classification integration was used on shape groups from PCA. Two hypotheses were developed. First, degree of shape groups' overlap had to be sufficient to warrant reuniting or dissolution of those shape groups from PCA. Second, specimen assignment to a given shape group had to be of a low enough degree to warrant expulsion from that shape group. Fuzzy measures were based on various combinations of head pole width, foot pole width, and mid-valve width (Fig. 1) as ratios to form ordered membership functions on the interval [0,1]. *Asterionella* shape is interdependent with morphometric measurements of head pole, foot pole, and mid-valve width. Ratios based on morphometric measures may be viewed as proxies for particular stages in a life cycle as size reduction occurs.

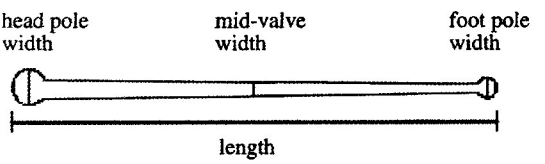

Fig. 1. Idealized *Asterionella* in valve view. Head pole width, mid-valve width, foot pole width, and length are indicated.

Classification integration includes a measurable function, h, and an importance measure, g (Wang & Klir 1992). The solution to Sugeno's (or the fuzzy) integral (Sugeno 1977) is the evaluation, E, given as

$$E = \oint h(x) \circ g(\bullet) = \oint f \, d\mu(x)$$

where $h(x)$, a measurable function represents partial evidence (degree of certainty) and $g(\bullet)$ is an importance measure (Sugeno 1977, Wang & Klir 1992). From fuzzy set theory, the solution to the fuzzy integral is

$$f[h(x) \circ g(\bullet)] = \bigvee_{i=1}^{n} [h(x_i) \wedge g(x_i)]$$

where $\{x_1, \ldots, x_n\}$ defines combinations of head pole width, foot pole width, mid-valve width, and $\vee$ and $\wedge$ are maximum and minimum operators, respectively.

For the first hypothesis, $h(x)$, a fuzzy average overlap is

$$\underset{\substack{x\in S_i \\ x\in S_l}}{h(x_i)} = \frac{1}{m+q}\left\{\sum_{j=1}^{m}\sum_{k=1}^{q}\mu_t(x_j)\mu_t(x_k)\right\}$$

where m is the number of elements in shape group S_i and q is the number of elements in shape group S_l (Dunn 1977, Terano *et al.* 1992). Partial evidence is based on membership function, μ_t, which equals the ratio of mid-valve width to head pole width.

Importance measure, $g(\bullet)$ was calculated as a possibility measure, $\prod$ (Wang & Klir 1992), and based on the ratio of foot pole width to head pole width. For shape groups S_i and S_l for possibility distributions π_{S_i} and π_{S_l}, respectively (Dubois & Prade 1980), and using the extension principle (Zadeh 1975, Dubois & Prade 1980),

$$\underset{\substack{x\in S_i \\ x\in S_l}}{g(x) = \pi(x) = \sup}\ \min\left(\pi_{S_i}, \pi_{S_l}\right)$$

where *sup* is the supremum or least upper bound (Dubois & Prade 1980, Kandel 1982, Zimmerman 1991, Wang & Klir 1992) and *min* is the minimum operator (Dubois & Prade 1980).

At 0.5, the crossover point, judgements about E may be made (Wang & Klir 1992). A non-overlap/overlap grade on [0, 1] is used whereby the threshold value is 0.5 (Dubois & Prade 1980).

For the second hypothesis, partial evidence is calculated as a ratio of normalized mid-valve width to foot pole width for each specimen. Importance measure is calculated as Sugeno's measure (Wang & Klir 1992) for two attributes. The first, g_1, is ratio of foot pole width to head pole width. The second, g_2, is ratio of mid-valve width to head pole width.

Sugeno's measure is

$$g_\lambda(X) = \frac{1}{\lambda}\left[\prod_{i=1}^{n}(1+\lambda g_i) - 1\right]$$

where $\lambda > 0$ defines g as a belief measure, $-1 < \lambda < 0$ defines g as a plausibility measure, and $\lambda = 0$ defines g as a probability measure (Dubois & Prade 1980, Wang & Klir 1992).

Again, at the crossover point, judgements about E may be made (Wang & Klir 1992). An exclusion/inclusion grade on [0, 1] is used whereby the threshold value is 0.5 (Dubois & Prade 1980).

Results

A number of PCAs were accomplished to determine shape groups. From the first PCA of 96 specimens, the first eigenvalue at 0.963 indicates that most of the shape variance is explained on the first eigenvector. With the second eigenvalue at 0.019, the first two eigenvectors accounted for 98.2% of total shape variance (Fig. 2). High positive correlations between shape coefficients and the first principal component (PC) ranged from 0.9313 to 0.9981. PC2 component loadings ranged from -0.3025 to 0.2487.

PC1 depicts maximum shape variance. The more common shaped *Asterionella* were farthest from the origin in the ordination. Based on geometric properties of head pole and foot pole within PCA ordination, seven shape group boundaries were drawn (Fig. 2).

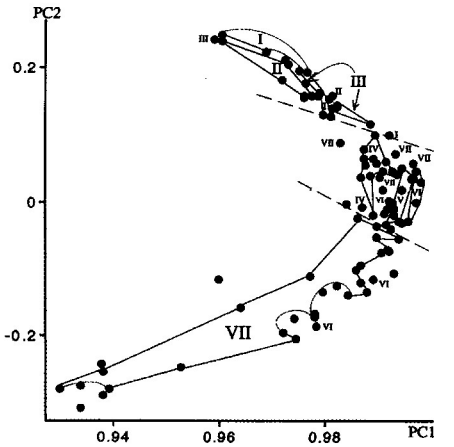

Fig. 2. PCA ordination of 96 *Asterionella* specimens' shape coefficients. Shape groups are labelled I - VII; some specimens were not assigned to shape groups.

The second PCA was accomplished using mean-corrected amplitudes from shape groups I-VII. Thirteen specimens were removed from analysis since they were not assigned to shape groups I-VII. From this PCA of 83 specimens, eigenvalues for the the first and second eigenvectors were 0.967 and 0.17, respectively. This accounted for 98.4% of total shape variance, which is approximately the same as that for the first PCA (Fig. 3). The seven shape groups were more clearly defined in this PCA.

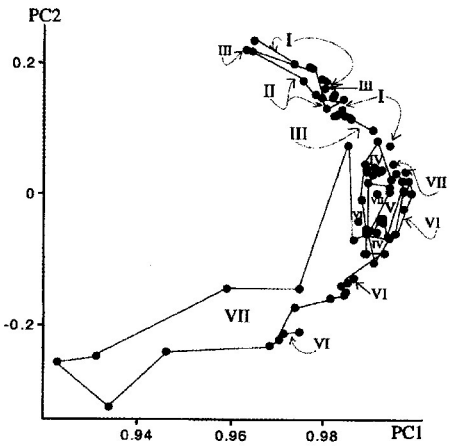

Fig 3. PCA ordination of 83 *Asterionella* specimens' shape coefficients. Shape groups are labelled I - VII.

The third PCA was used on mean-corrected amplitudes shape groups I-VI specimens. These shape groups were more difficult to differentiate. For the first two eigenvectors, eigenvalues were 0.976, and 0.011, respectively, accounting for 98.8% of total shape variance (Fig. 4). In this PCA, each shape group was more easily seen. Shape groups I-

III appear to be elongated and twisted around each other than shape groups IV-VI. Shape group IV was well defined, while shape groups V and VI were somewhat more intertwined (Fig. 4).

Two more PCAs were accomplished. One PCA was used only with regard to 24 specimens in shape groups I-III. The other was used only with respect to 35 specimens in shape groups IV-VI. PCA of shape groups I-III produced eigenvalues of 0.984 and 0.007 for the first and second eigenvectors, respectively. Total shape variance accounted for by the first two eigenvectors was 99.1%. Overlap of shape groups II and III was evident, while shape group I had two distinct subgroups (Fig. 5).

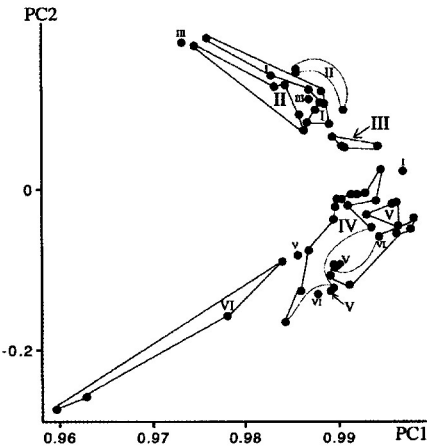

Fig 4. PCA ordination of 59 *Asterionella* specimens' shape coefficients. Shape groups are labelled I - VI.

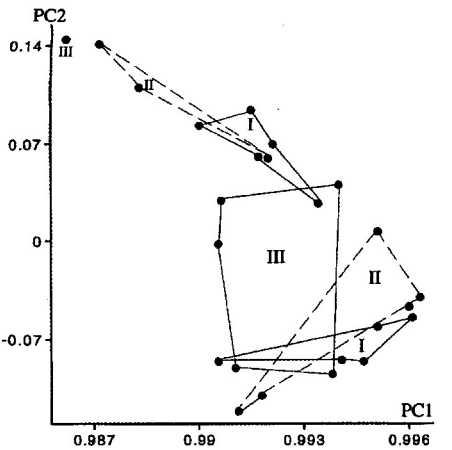

Fig 5. PCA ordination of 24 *Asterionella* specimens' shape coefficients. Shape groups are labelled I - III.

For shape groups IV-VI, PCA eigenvalues were 0.984 and 0.005 for the first and second eigenvectors, respectively, which represented 98.9% of total shape variance. There was some overlap among these groups. However, shape groups IV and V had a much greater degree of overlap (Fig. 6).

Each shape group may be described qualitatively. Shape group I included ten specimens that had wedge-shaped head and foot poles (Fig. 7). They also had an approximately 2:1 ratio of head to foot pole. In shape group II, eight specimens were included, and this group had wedge-shaped head and foot poles with the head poles being slightly more rounded in contrast to the first shape group (Fig. 8). Head to foot pole ratio was approximately 3:2. Six specimens comprised shape group III. These specimens had round head poles and mostly rounded foot poles (Fig. 9). Head to foot pole ratio was 3:2.

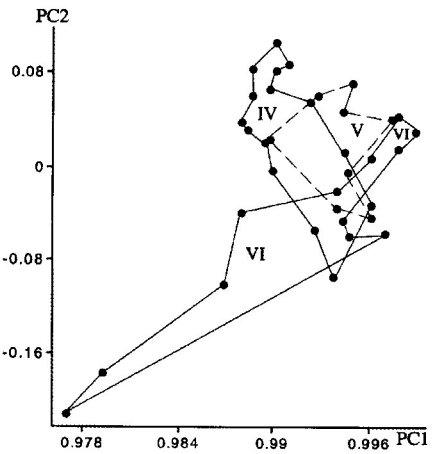

Fig 6. PCA ordination of 35 *Asterionella* specimens' shape coefficients. Shape groups are labelled IV - VI.

Shape group IV had fifteen specimens. These specimens had round head poles with the foot pole inflation barely discernable from the valve width (Fig. 10). For the most part, head to foot pole ratio was 3:2 with some specimens having a ratio of 2:1. Eight specimens comprised shape group V which had round head and foot poles (Fig. 11). Head to foot pole ratio was from 5:3 to 4:3. Shape group VI included twelve specimens. Head and foot poles were round, and they had very narrow mid-valve widths compared to head to foot pole ratio (Fig. 12). Mid-valve widths were mostly approximately 2 μm, while head to foot pole ratio was mostly 5:2 or 2:1.

Twenty-four specimens comprised shape group VII. Specimens in this shape group had an asymmetrical head pole which is 'golf-club shaped' (Fig. 13). The foot pole was round. Head to foot pole ratios were 6:2 for the longest specimen and approximately 1:1 for the smallest specimen. Most had a ratio of 5:2, 4:3 or 2:1.

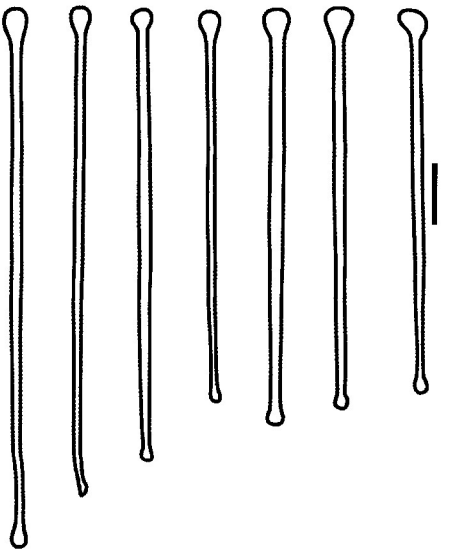

Fig 7-13. Outlines representing shape groups I-VII, respectively. Scale bar = 10 μm.

Two tests using fuzzy analysis of shape groups was accomplished. The first test was applied to paired combinations of shape groups I-III with respect to degree of overlap. Similarly, this test was also applied to shape groups IV-VI. Solution to the fuzzy integral as well as *h(x)* and *g(x)* were calculated for each shape group combination. Shape groups I and II had an evaluation value, *E*, at just above the crossover point. Shape groups II and III as well as shape groups I and III had evaluation values, *E*, at greater than the crossover point, but did not exceed the three-quarter point or 0.75 (Table 1). For each combination of shape groups IV, V, and VI, evaluation values, *E*, were just over the crossover point (Table 2). Values of *h(x)* for shape groups I-VI ranged from approximately 0.5 to 0.6 (Tables 1, 2). For shape groups I-VI, *g(x)* values were in approximately the same range as *h(x)* values (Tables 1, 2).

Table 1. Calculated values of *E* for combinations of shape groups I, II and III.

paired combination	*h(x)*	*g(x)*	*E*
$\mu_{I, II}(\{x\})$	0.49	0.63	0.53
$\mu_{II, III}(\{x\})$	0.57	0.60	0.60
$\mu_{I, III}(\{x\})$	0.52	0.57	0.60

Table 2. Calculated values of *E* for combinations of shape groups IV, V and VI.

paired combination	*h(x)*	*g(x)*	*E*
μIV, V({x})	0.55	0.59	0.56
μV, VI({x})	0.47	0.46	0.55
μIV, VI({x})	0.50	0.46	0.56

The second test was used to determine degree of membership for each specimen. Parameter λ was calculated to determine whether results exhibited degree of belief or degree of plausibility for each shape group. Values for parameter λ ranged from approximately -0.6 to -0.9 indicating plausibility of results (Table 3).

Table 3. Values for g_λ - fuzzy measures, g_1 and g_2, and λ for shape groups I-VI.

SHAPE GROUP	g_1	g_2	λ
I	0.68	0.52	-0.5656
II	0.69	0.67	-0.7787
III	0.60	0.73	-0.7534
IV	0.75	0.73	-0.8767
V	0.72	0.61	-0.7514
VI	0.63	0.68	-0.7236

A solution to the fuzzy integral was calculated for each specimen for each shape group. Specimens from shape group I had evaluation values, E, of approximately 0.6 to 0.7 (Table 4). For shape group II, evaluation values, E, ranged from slightly less than 0.6 to just over 0.65 (Table 4). All specimens from shape group III had evaluation values, E at 0.6 (Table 4).

Table 4. $E(x_I)$, $E(x_{II})$, and $E(x_{III})$ values for each *Asterionella* specimen.

specimen	$E(x_I)$	specimen	$E(x_{II})$	specimen	$E(x_{III})$
1997-8	0.62	1241-7	0.57	1447-2	0.60
1180-1	0.68	1997-7	0.60	1180-8	0.60
1997-10	0.68	1180-13	0.61	1976-7	0.60
1997-3	0.68	1341a-4	0.66	1976-4	0.60
1539a-1	0.68	1180-5	0.67	1976-11	0.60
1997-5	0.68	1241-9	0.67	1976-8	0.60
1997-14	0.68	1505-5	0.67		
1997-13	0.68	1505-2	0.67		
1997-2	0.68				
1180-6	0.68				

Table 5. $E(x_{IV})$, $E(x_V)$, and $E(x_{VI})$ values for each *Asterionella* specimen.

specimen	$E(x_{IV})$	specimen	$E(x_V)$	specimen	$E(x_{VI})$
1997-1	0.61	1447-9	0.61	1312-1	0.49
1309-6	0.68	1997-9	0.61	1312-12	0.63
1241-11	0.72	1447-1	0.61	1241-10	0.63
1341a-3	0.73	1341a-2	0.61	1997-4	0.63
1309-16	0.73	1447-3	0.61	1309-1	0.63
1312-5	0.73	1180-2	0.61	1309-2	0.63
1447-8	0.73	1241-6	0.61	1309-3	0.63
1447-15	0.73	1447-12	0.61	1312-4	0.63
1241-3	0.73			1312-9	0.63
1312-10	0.73			1447-4	0.63
1341a-11	0.73			1309-4	0.63
1447-13	0.73			1997-6	0.63
1341a-5	0.73				
1341a-6	0.73				
1309-7	0.73				

Shape group IV specimens had evaluation values, *E*, from approximately 0.6 to slightly larger than 0.7 (Table 5). In shape group V, all specimens had evaluation values, *E*, equal to 0.61 (Table 5). Evaluation values, *E*, for shape group VI specimens were equal to 0.63 except one which was at 0.49 (Table 5).

Discussion

Complexity of morphological variation is difficult to assess on an exclusively empirical basis (Mann 1999). For a character-poor diatom, such as *Asterionella*, this is especially true with respect to valve shape. Difficulties arise in documenting shape change throughout the diatom life cycle. Quantitative shape analyses provides a way to define individual life cycle stages which may be related to ontogeny of valve shape. Fourier coefficients from orthogonal polynomial regression were used as quantitative representations of valve shape. Measurable or quantifiable variation (rather than qualitative differences) within the context of all phenotypic variation is a result of physiological processes having more of a genetic rather than environmental influence (Pappas & Stoermer 1995)

Size diminution is a physiological process which is influenced in many ways. *Asterionella* is known to have complex physiological variation with respect to mophology (Happey-Wood & Hughes 1980). Diatom shape at different life cycle stages is influenced primarily by size diminution (Schmid 1994, Mann 1994). Although some change in shape occurs from turgor pressure and mitotic division, shape restoration occurs as a result of mitotic division after auxosporulation (Schmid 1994, Mann 1994). Diatoms exhibit a wide range of variation in form and pattern reflecting range of physiological adaptations with respect to turgor stresses on the cell. Size reduction from mitotic cell division not only defines species-specific shape, but also means that interspecies valve shape changes cannot occur (Mann 1994). Species-specific form and structure are variable within a genetical framework and is dependent on the interrelation between the diatom cell and environment (Schmid 1979).

From a series of PCAs, seven shape groups were determined and tested using fuzzy analysis. In the first two PCAs (Figs 2, 3) from seven subdivisions, shape group VII had the largest number of members. In fact, there was approximately an entire size series for this group whereby the shortest specimen was almost 30% the size of the longest specimen (Edlund & Stoermer 1997). Ontogenetic properties of diatom shape (Mann 1994) would suggest that members of shape group VII may be considered to be an *Asterionella* species separate from the remaining specimens analyzed.

In valve development, there is an ontogenetic order (Williams 1990). In *Tetracyclus*, another araphid taxon, ontogeny of shape was determined from cells at post-auxospore stage to those which were about to undergo auxosporulation, thereby covering the entire size diminution process. In general, valve form changed from constriction or inflation in the central area to an elliptical or circular form at some point in the life cycle of *Tetracyclus* Ralfs.

Modern and fossil populations were examined (Williams 1990, 1996). For *T.*

constrictus (Ehrenberg) De Toni (Williams 1996) and *T. javanicus* Hustedt (Williams 1990, 1996), which have constrictions, valve shape changed to an elliptical form just prior to auxosporulation. For *T. lata* (Hustedt) D.M. Williams *nov. stat.* and *T. 'chilensis'* (taxa resembling and including *T. ellipticus* var. *lacea* f. *chilensis* Krasske) constriction changed to an elliptical form much earlier in the life cycle (Williams 1996). At the pre-auxospore stage, *T. lata* became circular, and *T. 'chilensis'* remained elliptical. *Tetracyclus glans* (Ehrenberg) Mills and *T. stella* (Ehrenberg) Héribaud have central inflations whereby valve shape change to elliptical form occurred at the pre-auxospore stage (Williams 1996). In all *Tetracyclus* life cycles, elliptical stage occurred as an ontogenetic property. Particular valve shapes occur at particular life cycle stages. The elliptical shape of *Tetracyclus* is a variable ontogenetic property (Williams 1996).

In *Asterionella* shape group VII, specimens became wider as they became shorter (Fig. 14). In the smallest specimens, head pole and foot pole becomes less distinguishable from the central linear part of the valve. As specimens decrease in length, they become more rounded with less distinct outlines as exhibited by many diatoms (Geitler 1932). The asymmetrical head pole remains distinct until the very late stages depicted in the size reduction series (Fig. 14). The smallest specimen has a much less distinct head pole. If shape group VII size series represents life cycle stages, inflated ends with the head pole being asymmetrical becomes less inflated ends with the head pole losing much of its asymmetry.

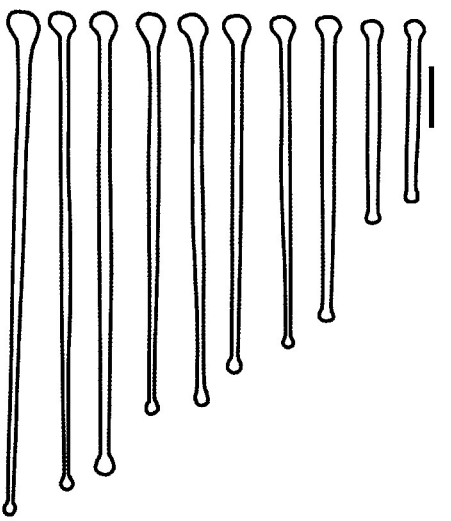

Fig. 14. Outlines of size series for shape group VII. Scale bar = 10 µm.

In shape groups I-VI, specimen lengths did not encompass the entire size range. All specimens from shape group I were from 90 to 96 µm in length. In shape groups II, and III, the smaller specimens were approximately 75 to 80% the length of the longest

specimens. Shape groups IV, V, and VI had smaller specimens 60 to 70% the length of the longest specimens. According to Mann (1988), some *Asterionella* maintain a constant size such as *A. formosa* var. *'hypolimnetica,'* while others undergo size reduction, including other *A. formosa* Hassall varieties and *A. gracillima* (Hantzsch) Heiberg. Some may undergo rapid size reduction (Kling 1993). Even with taxa where size reduction has been demonstrated, auxosporulation has not been documented for *Asterionella* species. For *A. gracillima* in seven Danish lakes, size series of specimens found from 1900 to 1906 ranged from 38 to 100 μm in length with a rate of reduction at 2.5 μm per year. This results in a projected 20 year life cycle for *A. gracillima* in these lakes (Mann 1988). With a long life cycle, observing auxosporulation or obtaining specimens which encompass an entire size range is difficult. Lack of entire size series makes judgments about ontogeny of valve shape in shape groups I-VI difficult.

The degree that *Asterionella* separated into particular shape groups revealed to some extent shape variation in Great Lakes specimens. In particular, *Asterionella* shape variation in shape groups I-VI reflected this. PCA depicted shape groups I, II, and III forming a subgroup distinct from shape groups IV, V, and VI. Ordination of shape groups I-VI emphasized this separation as well as showing the relation among shape groups I-III and the relation among shape groups IV-VI (Fig. 4). Shape group I was distinct from and split shape group II Fig. 4). Shape group IV was distinct from and split shape group VI (Fig. 4). Shape groups III and V were less well defined, and each may represent having some affinity for shape groups I or II and shape groups IV or VI, respectively.

This was depicted with additional PCAs. PCA of shape groups I-III showed the relation among these groups more readily (Fig. 5). Shape group III overlapped more with shape group II than with shape group I (Fig. 5). PCA of shape groups IV-VI revealed the interrelation of these groups (Fig. 6). Shape group V overlapped more with shape group IV than with shape group VI (Fig. 6). If shape groups I and II are separate species, shape group III may represent a subspecies or variety. The same may be true of shape group V with respect to shape groups IV and VI.

Fuzzy analysis of the shape groups created and the individual specimens that were assigned to the groups reflected the uncertainties associated with determining how much shape variation constitutes biological separation. That is, how similar in valve outline is sufficient to constitute grouping particular specimens into a single species (Round *et al.* 1990)?

Paired combinations of shape groups I-III and IV-VI resulted in evaluation, E, values which did not support degree of overlap over 0.60. This indicated that separation of shape groups is partially supported to the degree of at least 0.40. That is, some differences do exist among shape groups.

For paired combination of shape groups I and II, $E_{I,II}$ at 0.53 indicated that approximately the same degree of overlap is equal to the degree of non-overlap (Table 1). Since overlap was not at least at the three-quarter point or greater, there is not sufficient enough reason to require that shape groups I and II are one and the same

biological entity. Value of *g(x)* indicates high possibility of overlap (0.60); however, degree of certainty, *h(x)*, is at the crossover point (Table 1). Shape groups I and II may represent different subspecies or varieties or forms of the same species.

For paired combination of shape groups II and III, $E_{II,III}$ is a 0.60 (Table 1). The same value was determined for paired combination of shape groups I and III (Table 1). However, partial evidence value, *h(x)*, was highest for paired combination of shape groups II and III. For paired combination of shape groups II and III, *g(x)* was at 0.60. The results indicated a greater possibility [*g(x)*] and a greater degree of certainty [*h(x)*] that shape groups II and III overlap to degree 0.60 (*E*) (Table 1). Shape groups II and III may represent the same species, subspecies, variety, or form. With *h(x)* at approximately the crossover point, shape groups I and III may represent different subspecies or varieties or forms of the same species.

Paired combinations of shape groups IV, V, and VI showed that shape groups IV and V had a greater possibility [*g(x)* at 0.59] and a greater degree of certainty [*h(x)* at 0.55] that they overlap with $E_{IV,V}$ at 0.56 (Table 2). These shape groups may represent the same species, subspecies, variety or form. Similar results were not the case for paired combinations of shape groups V and VI as well as shape groups IV and VI (Table 2). Although $E_{V,VI}$ and $E_{IV,VI}$ were about the same as that for $E_{IV,V}$, *h(x)* and *g(x)* are only at or just below the crossover point (Table 2). That is, there is less possibility and certainty of overlap. Results from the pairings of these shape groups may represent different subspecies, varieties, or forms of the same species.

Of the tested pairings, only paired combination of shape groups II and III as well as shape groups IV and V exhibited greater certainty in degree of overlap. Evaluation values of $E_{II,III} = 0.6$ and $E_{IV,V} = 0.56$ were somewhat greater than the crossover point. Perhaps this indicated misassignment of some specimens in each group as well as overlap. This led to the second test using fuzzy analysis.

Degree of membership for each specimen in assigned shape groups was also tested by fuzzy analysis. Results from Sugeno's measure and parameter λ showed that all specimen assignments were plausible (Table 3). The only exception may be g_2 for shape group I which was approximately at the crossover point (Table 3). Three values for either of Sugeno's measure were at approximately 0.60 (shape groups III, V, and VI). Most values were at approximately 0.70 with one value at 0.75 (for shape group IV). Shape groups II and IV had the highest values for both of Sugeno's measures (Table 3).

In general, *h(x)* values were similar to results for *E*. Evaluation values generally exceeded the crossover point which indicated degree of inclusion for each member from shape groups I-VI. All but two values of *E* for each specimen were in the range of 0.60 to 0.73. That is, membership for these specimens in their assigned shape group was at least 0.60 which well exceeds the crossover point. From shape group II, one member had a value of *E* at 0.57, which may be considered questionable if the value of *E* is not rounded up (Table 4). Members of shape group IV had the highest values of *E* with all but one member at approximately 0.70 (Table 5). One member of shape group VI had a value of *E* at just under the crossover point (Table 5). This may be considered

an equivocal assignment. Overall, specimen assignments were acceptable to at least degree 0.60, and there is no clear example of exclusion from any shape group (where values were at well less than the crossover point).

Fuzzy assessment of *Asterionella* shape groups indicates that while distinct shape groups are evident, separation is not complete. Despite some overlap, shape groups I-III as well as shape groups IV-VI are different to some degree since overlap is not complete. The degree to which these differences indicate biologically differences may help define species, subspecies, varieties, or forms. Boundaries between variation patterns which are hierarchical or above species level and non-hierarchical or below species level are fuzzy (Mann 1999). Fuzzy boundaries between shape groups as biological separation may be defined as non-hierarchical if each group represented a subspecies, variety or form.

Some evidence exists to suggest that genetic differences are present within *Asterionella*. Isolates of *A. formosa* from thirty freshwater systems were analyzed to determine population structure (Soudek & Robinson 1983). This included populations from Lakes Michigan and Superior. From the centroid method of hierarchical cluster analysis, seven clusters were found of population-specific electrophoretic banding patterns. These results included separation of the Lake Michigan population from the Lake Superior population. Slight genetic differences were found between isolates from Lake Superior at Rossport and those from the St. Mary's River. Large lakes may have more than one phenotype restricted to different basins (Soudek & Robinson 1983). Different banding patterns for specimens from different lakes were evident, despite one lake emptying into another lake. Definite genetic differences were determined to exist among populations of *Asterionella* from different lakes (Soudek & Robinson 1983). *Asterionella formosa* was observed to have a lack of genetic heterogeneity. That is, there is little to no genetic variation within populations in contrast to genetic variation between populations (Soudek & Robinson 1983). Most of the specimens in shape groups I-III were from the northern Great Lakes, while the majority of specimens in shape groups IV-VII were from central and southern Lake Michigan. From genetic evidence (Soudek & Robinson 1983), there may be a difference in genetics among the larger shape groups determined in our study.

Shape group separation was not unequivocal in our study. However, some degree of shape differences were determined among groups, and these differences were partially highlighted by proxies for size reduction stages used in fuzzy analysis. In addition, our study showed that *Asterionella* taxonomy requires ongoing work with many more specimens. It would be helpful to find specimens to fill out the size ranges (if available) of shape groups I-VI so that the same tests may be applied. As a result, better shape group separation or consolidation may occur. Approaching taxonomy from the viewpoint of valve shape ontogeny may be one of the better ways to study character-poor *Asterionella*. There is a continuing need to document the sequence of events relating valve shape to vegetative reproduction and frustule formation (Crawford 1981) to improve taxonomic studies of *Asterionella*.

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