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Article

Geometric morphometrics of symmetry and allometry in *Micrasterias rotata* (Zygnemophyceae, Viridiplantae)

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With 6 figures

Abstract; Algal cells show many types of complex symmetry. For instance, *Micrasterias rotata* cells are symmetric relative to two perpendicular axes of symmetry. Due to the mode of cell division of *Micrasterias rotata* cells, the symmetry axes can be interpreted as a left-right axis and a juvenile-adult axis. Here, we analyze symmetry and allometry in *Micrasterias rotata* cells. We apply a new general approach for shape analysis of structures with any type of symmetry. Our method can separate a component of symmetric variation among individuals from one or more components of asymmetry, depending on the type of symmetry under study, and thus is appropriate for the study of symmetry in these cells. Our results suggest that almost two-thirds of the shape variation in our example (62.3 % of the total variance) is explained by the asymmetric component relative to the juvenile-adult axis. Therefore, most morphological variation occurs between juvenile and adult semicells. Given that these shape changes are associated with the size of the semicells, they indicate a type of allometry between semicells. We also compared these patterns of allometry within cells to allometry among cells by using a multivariate regression of shape averaged by individuals onto size of the whole cells. A permutation test shows a highly significant association between size and shape of the whole cells.

Key words: *Micrasterias rotata*, geometric morphometrics, Procrustes superimposition, shape analysis, symmetry, morphological variation, allometry

Introduction

Algal cells show a great morphological diversity and exhibit many intricate patterns of symmetry. For instance, many algal cells are radially symmetric (e. g., valve view of *Cyclotella meneghiniana*, Bacillariophyta) while others are symmetric with respect to two perpendicular axes of bilateral symmetry (e. g., valve view of *Achnanthidium minutissimum,* Bacillariophyceae). In *Micrasterias rotata*, the fully grown cells are symmetric relative to two perpendicular axes of symmetry. One axis passes through the isthmus and divides the cell into a juvenile and adult semicells, the second separates the left and right sides of the semicells. These cells are a unique biological system that allows the investigation of complex symmetry combined with a very interesting growth (e. g., Meindl 1993).

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Recently, Potapova & Hamilton (2007) have presented a method for shape analysis of symmetry in diatoms that are symmetric with respect to two perpendicular axes of bilateral symmetry and only the symmetric part of the variation was considered. Frey et al. (2007) also designed a framework for analysis of rotational symmetry only. We developed a more general method for shape analysis of all possible types of symmetry (Savriama & Klingenberg 2006, Savriama & Klingenberg subm.) and we apply this approach to data collected from a clonal culture of *Micrasterias rotata* cells. This framework can distinguish a component of symmetric variation from one or more components of asymmetric shape changes that might occur depending on the type of symmetry under study and therefore is suitable for a complete study of symmetry in *Micrasterias rotata* cells.

Given that the cells undergo a particular type of growth that implies considerable ontogenetic differences between juvenile and adult semicells within a single cell, we suppose these growth differences may induce more morphological variation between the juvenile and adult semicells than between the left and right halves of the semicells. Also, juvenile semicells are smaller than the adult semicells and the morphological differences between them may be related to differences in size. That means there is a type of allometry in the differences between semicells, which can be analyzed as a component of variation within cells. We also test for allometry among cells to compare the shape changes associated to variation in size between semicells and allometry of the whole cells.

Materials and methods

Specimens and data collection

We used the K604 strain of *Micrasterias rotata* cells from the Culture Collection of Algae of Charles University of Prague (CAUP). Cells were grown in identical conditions at 25 °C, in 12:12 hours day/night illumination and in CAUP oligotrophic medium (see at http://botany.natur.cuni.cz/algo). In total, 58 cells were photographed at the same magnification with an Olympus BX51 light microscope. For each photograph, 20 landmarks (Fig. 1) were digitized in two dimensions using tpsDig version 2.05 (Rohlf 2006). In both juvenile and adult semicells, landmarks 1, 9, 18 and 10 describe the lower extremities of lower lateral lobules, landmarks 2, 8, 17 and 11 represent the bases of incisions between the lower lateral lobules, landmarks 3, 7, 16 and 12 pinpoint the bases of incisions between polar and lateral lobes, landmarks 4, 6, 15 and 13 identify the lateral margins of the polar lobes, landmarks 5 and 14 locate the incision of the polar lobes and landmarks 19 and 20 represent the margins of the isthmus.

Definition of symmetry

An object is symmetric if it remains unchanged after a set of transformations is applied to it (e. g., Weyl 1952). For example, the human face is symmetric because it remains identical after a reflection about its middle axis (or plane). The simplest transformation is the identity, which maps any point onto itself. Other transformations include rotations, reflections and translations. The transformations that leave an object unchanged are called symmetry transformations. Every type of symmetry is associated with a set of symmetry transformations. This set forms a mathematical group, which is called the symmetry group (e. g., Rosen 1975). For instance, bilateral symmetry is associated with a symmetry group that contains two transformations: the identity and a reflection. For the *Micrasterias* cells in this study, the symmetry group contains four transformations (Fig. 2).

Some symmetry groups are finite because they contain a countable number of symmetry transformations; all of these symmetry groups can be generated by combining reflections and/or rotations

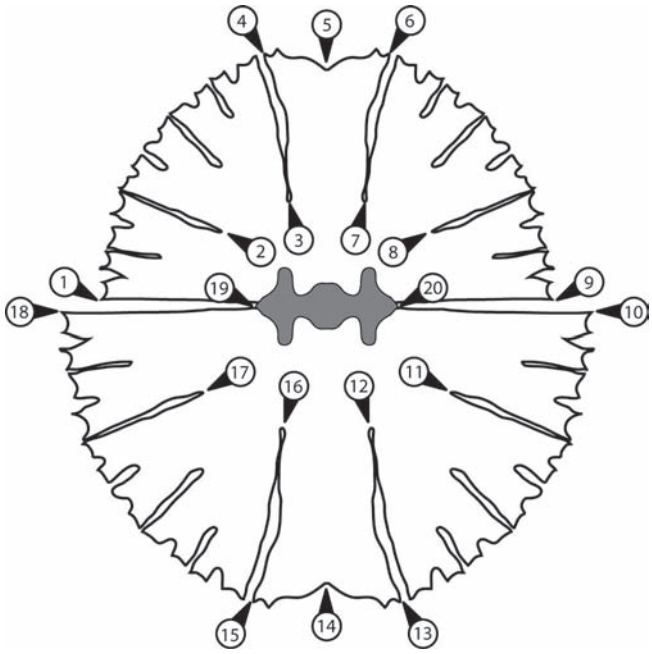

Fig. 1. Schematic representation of a cell of *Micrasterias rotata* with a less developed juvenile semicell (upper half) and a more developed adult semicell (lower half). The landmarks used in this study are also shown. Note that the cell structure and arrangement of landmarks are symmetric about the horizontal and vertical axes, except for asymmetries such as the difference in growth between the juvenile and adult semicells.

(e. g., Rosen 1975). A mathematical definition of symmetry using the theory of symmetry groups provides a standardized and systematic approach to the study of symmetry.

Shape analysis of symmetry

The analysis of shape in geometric morphometrics is done with multivariate statistical analyses performed on landmark coordinates after discarding the extraneous information of size, position and orientation (e. g., Dryden & Mardia 1998). This extra information is eliminated using a generalized Procrustes fit according to a least-squares criterion. A mean shape configuration (consensus) is computed and variation around this mean can be calculated (e. g., Dryden & Mardia 1998).

By combining the reasoning based on symmetry groups and the methods of geometric morphometrics, it is possible to analyze shape variation in any symmetric object. First, the original configuration of landmarks for the whole structure is duplicated as many times as there are symmetry transformations in the symmetry group of the object. Then, these copies are included in a new dataset and the symmetry transformations in the symmetry group are applied to them (Fig. 2). Finally, a joint Procrustes fit is applied to the combined data set.

If there is reflection in the symmetry group, the configuration of landmarks consists of a combination of paired landmarks that are located outside of the axis of symmetry and unpaired landmarks that are aligned on it. Reflected copies of these configurations are generated by multiplying one of the coordinates of all landmarks by –1 (e. g., all x-coordinates). To include the original and

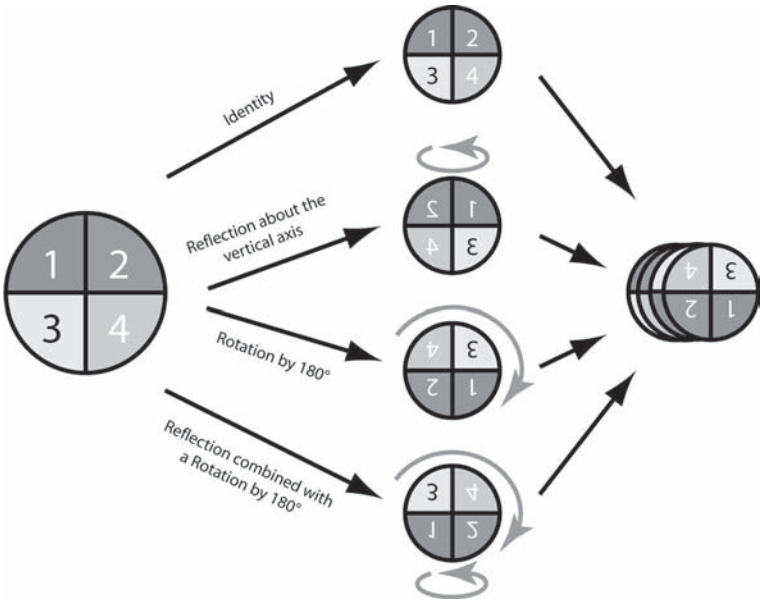

Fig. 2. Analysis of a symmetric structure with two perpendicular axes of bilateral symmetry. The symmetry group of the object contains four symmetry transformations and therefore is finite: the identity, reflection about the vertical axis, a rotation by 180° (which is identical to two successive reflections about both axes) and a combination of reflection with rotation by 180° (which is equivalent to reflection about the horizontal axis). An original configuration of landmarks and all its transformed copies with the landmarks appropriately relabelled are superimposed simultaneously by a Procrustes fit. The resulting consensus shape is symmetric.

reflected copies of the landmark configuration in combined dataset together, it is necessary to relabel the landmarks in the reflected copies by interchanging the corresponding paired landmarks on either side of the axis of symmetry (Mardia et al. 2000, Klingenberg et al. 2002).

If the symmetry group contains rotations, the whole configuration can be represented as sectors arranged around the centre or axis of rotation (like a pie cut into a number of equal slices). Rotated copies are produced in a way that depends on the order of rotation, the number of repeated steps it takes to cover a full circle (i.e., for a rotation of order n, each step is a rotation by $360°/n$). Two types of landmarks can be distinguished: those at the centre or axis of rotation (which may be present or not) and landmarks outside the centre or axis of rotation that are present in each repeated sector. Rotated copies are produced by mapping the landmarks of each sector to the corresponding landmarks in the sector that is removed by a given number of steps. One rotated copy is needed per repeated step up to the order of rotation. Only this relabelling is needed to generate the rotated copies because the Procrustes fit will automatically perform the rotations to superimpose the corresponding landmarks of the rotated and relabelled copies.

In our case, the symmetry group of a *Micrasterias rotata* cell contains four symmetry transformations (Fig. 2): the identity, reflection about the left-right axis, rotation of order 2 (i.e., by 180°; this is equivalent to two successive reflections about the two axes), and the combination of reflection about the left-right axis and rotation by 180° (identical to reflection about the juvenile-adult axis).

Finally, a Procrustes fit simultaneously superimposes the combined dataset to extract shape information after discarding the extraneous information of size, position and orientation (e. g., Dryden & Mardia 1998). The mean shape configuration (consensus) from the Procrustes fit is

completely symmetric under all symmetry transformations in the symmetry group (for details see Savriama & Klingenberg 2006, Savriama & Klingenberg *submitted*).

We decompose the variation around the consensus into a component of symmetric variation and one or more components of asymmetry by calculating the appropriate averages or differences among the Procrustes coordinates obtained after the Procrustes fit. The component of symmetric variation is measured by the differences among the individual averages over all transformed copies and represents the variation among individuals. A component of asymmetry can be extracted from the differences among the transformed copies of each individual. Depending on the type of symmetry under study, multiple components of asymmetry may arise. For example, for *Micrasterias rotata* cells, which are symmetric with respect to reflection and rotations by 180°, there are three components of asymmetric variation: a component of variation that is asymmetric relative to reflection about the juvenile-adult axis but symmetric with respect to reflection about the left-right axis, a component of variation that is asymmetric with respect to reflection about the left-right axis but symmetric under reflection about the juvenile-adult axis, and a component of variation that is asymmetric under reflection about both axes but symmetric under rotations by 180°. Note that there is no totally asymmetric component for this type of configuration because of constraints imposed by the Procrustes fit.

Analyses

We apply our new method to the data collected from *Micrasterias rotata* cells. The combined data set of original landmark configurations and reflected and rotated copies is superimposed with a full Procrustes fit and the data are projected into the tangent space by orthogonal projection (Dryden & Mardia 1998).

In the shape analysis of bilateral symmetry, it has been demonstrated that after the landmark coordinates have been appropriately transformed, relabelled, and superimposed by a Procrustes fit, a principal component analysis (PCA) of the combined dataset can separate the components of symmetric variation from the components of asymmetric shape changes (Kolamunnage & Kent 2003). We extend the approach of Kolamunnage & Kent (2003) to separate the components of shape variation for a more complex type of symmetry.

To investigate if the shape changes are associated to variation in size of the whole cells, we use a multivariate regression of the Procrustes coordinates on centroid size (e. g., Monteiro 1999). Centroid size is the most common and explicit measure of size in geometric morphometrics, which is computed as the square root of the sum of the squared distances of all landmarks from their centroid (e. g., Dryden & Mardia 1998). To compute the component of symmetric variation, we averaged the Procrustes coordinates for the original and transformed copies of the landmarks of each individual. The relationship between the shape variables and centroid size in the multivariate regression can be visualized by plotting a shape score against centroid size (Drake & Klingenberg 2008). This shape score is a projection of each data point onto the direction of the regression vector in the shape tangent space, and is therefore the shape variable that is most closely related to centroid size (for details see Drake & Klingenberg 2008). The statistical significance of the relationship between the shape scores and the centroid sizes is assessed by a permutation test with 10,000 rounds of random permutations (e. g., Good 2000). All statistical procedures were carried out with MorphoJ (Klingenberg 2008) and the SAS system for Windows Version 9.1.3 (SAS Institute, Inc., Cary, NC).

Results

The PCA produced 36 principal components (PCs), which corresponds to the dimensionality of the shape tangent space (the total number of landmarks, 20, multiplied by 2 minus 4). The PCs can be divided into four categories according to the symmetry of the associated shape changes. Here, we present one PC for each category as an example (Fig. 3). The PC1 belongs to the first category and it is asymmetric with respect to reflection about the juvenile-adult axis but symmetric relative to reflection about the left-right axis (Fig. 3A). The patterns of shape variation for the PC1 show an overall reduction of the juvenile semicell compared to the adult semicell. For the juvenile semicell, landmark displacements occur towards the centre of the cell at the bases of incisions between lateral lobules, polar lobes and lateral lobes, whereas opposite movements

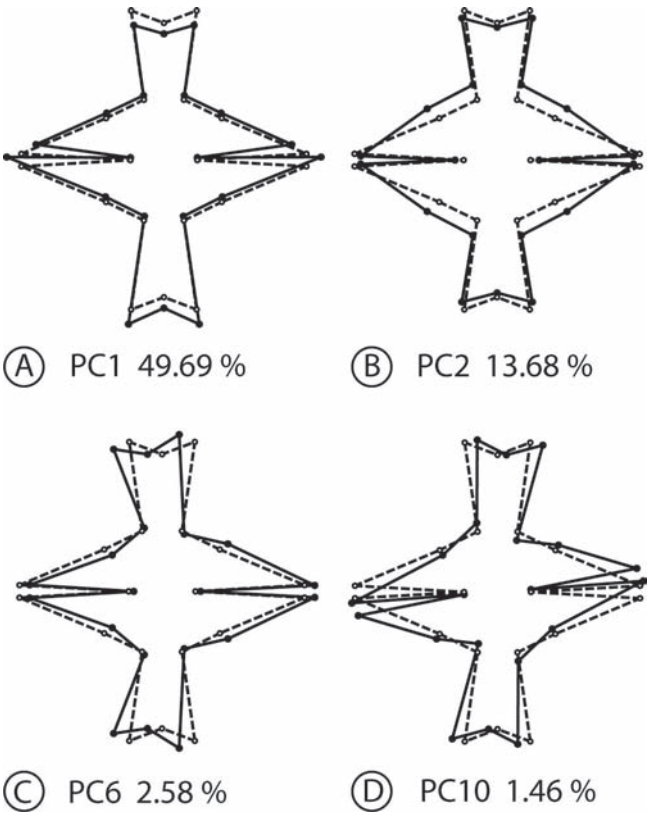

Fig. 3. Decomposition of shape variation in *Micrasterias rotata* cells. This figure shows examples of PCs for each category of shape variation. Each diagram shows the symmetric consensus (open circles and dotted lines) and the differences between the consensus and the other configuration (solid circles and solid lines) represent the shape change associated with the respective PC by an arbitrary amount of + 0.1 units of Procrustes distance. The percentages represent the part of the total shape variation for which each PC accounts. **A.** PC1. The shape change is asymmetric relative to reflection about the juvenile-adult axis but symmetric with respect to reflection about the left-right axis. **B.** PC2. This shape change is totally symmetric, i.e., symmetric under all transformations in the symmetry group. **C.** PC6. The shape change is asymmetric under reflection about the left-right axis but symmetric under reflection about the juvenile-adult axis. **D.** PC10. The shape change is asymmetric relative to reflection about both axes but symmetric under rotations by 180°.

take place in the adult semicell (Fig. 3A). Another category of shape variation is represented by the PC2, which is completely symmetric with respect to reflection and rotation by 180° (Fig. 3B). In this PC, the landmarks are shifting away from the centre of the cell at the bases of incisions between lateral lobules, polar lobes and lateral lobes. The PC6 belongs to another class of shape changes, which is asymmetric with respect to reflection about the left-right axis but symmetric relative to reflection about the juvenile-adult axis (Fig. 3C) and another group of shape variation is represented by the PC10, which is asymmetric with respect to reflection about both axes but symmetric relative to rotation by 180° (Fig. 3D).

The symmetries of the shape changes associated with PCs are key to interpreting the scatterplots of PC scores (Fig. 4 and 5). Because the shape change for the PC1 is symmetric under reflection about the left-right axis (Fig. 3A), this PC cannot differentiate according to this reflection and, as a consequence, the copies of a given cell's configuration that differ only by this reflection will have the same PC1 scores. Likewise, because the shape change for the PC2 is completely symmetric (Fig. 3B), all four copies for any given cell receive the same PC2 score. The plot of the PC1 versus PC2 scores reflects these properties: it shows a distribution of the PC scores that is symmetric under reflection about the vertical axis (Fig. 4). Each data point corresponds to two copies of the landmark configuration of the corresponding cell that differ by a reflection about the left-right axis, because the shape changes for both the PC1 and PC2 are symmetric under this transformation. However, because the PC1 does distinguish the shapes according to the reflection about the juvenile-adult axis, whether or not the copies of a given cell are reflected about that axis determines the sign of their PC1 scores. As a result, there is a symmetric scatter of points (Fig. 4).

The situation for the plot of the PC1 versus PC3 scores is somewhat different, because both these PCs are associated with shape changes that are symmetric under reflection about the left-right axis but asymmetric about the juvenile-adult axis. Because neither axis distinguishes the left-right reflections, copies of a given cell's landmark configuration that differ just by this reflection fall on the same point in the plot (Fig. 5). Because both the PC1 and PC3 pick up differences in reflection about the juvenile-adult axes, the scores of copies of a given configuration that differ in this reflection have opposite signs for both the PC1 and PC3, which in turn means that they are related by a rotation by 180° about the origin of the coordinate system. The result is that the scatter plot shows rotational symmetry of order 2 (Fig. 5).

Because the different types of PCs can be clearly identified, it is possible to add up the eigenvalues of PCs that belong to a specific category of shape variation to determine how much variance each category of shape changes account for. In total, 9 PCs are asymmetric relative to reflection about the juvenile-adult axis but symmetric with respect to reflection about the left-right axis and account for most of the shape variation (62.3 % of the total variance). The rest of the shape variation is taken up by 9 PCs that are totally symmetric under reflection and rotations by 180° (25.8 % of the total variance), 9 PCs that are asymmetric with respect to reflection about the left-right axis but symmetric regarding reflection about the juvenile-adult axis (6.1 % of the total variance) and 9 PCs that are asymmetric relative to reflection about both axes but symmetric under rotations by 180° (5.8 % of the total variance).

These results suggest that almost two-thirds of the morphological variation is explained by differences between adult and juvenile semicells. Given that the juvenile semicells are smaller than the adult semicells, the shape differences between semicells appear to be associated with size. Therefore, the patterns of shape changes related to growth obtained via the PCA can be viewed as a type of allometry that occurs between semicells of different age, in other words, a type of allometry within cells.

To compare these patterns of within-cell allometry to patterns of allometry among cells, we use a multivariate regression of the Procrustes coordinates averaged by individuals on centroid size of the whole cells. A plot of the shape scores from the multivariate regression against the centroid size shows a clear relationship between cell shape and size (Fig. 6A). Centroid size accounts for 19.49 % of the total amount of shape variation and the permutation test indicates that the multi-

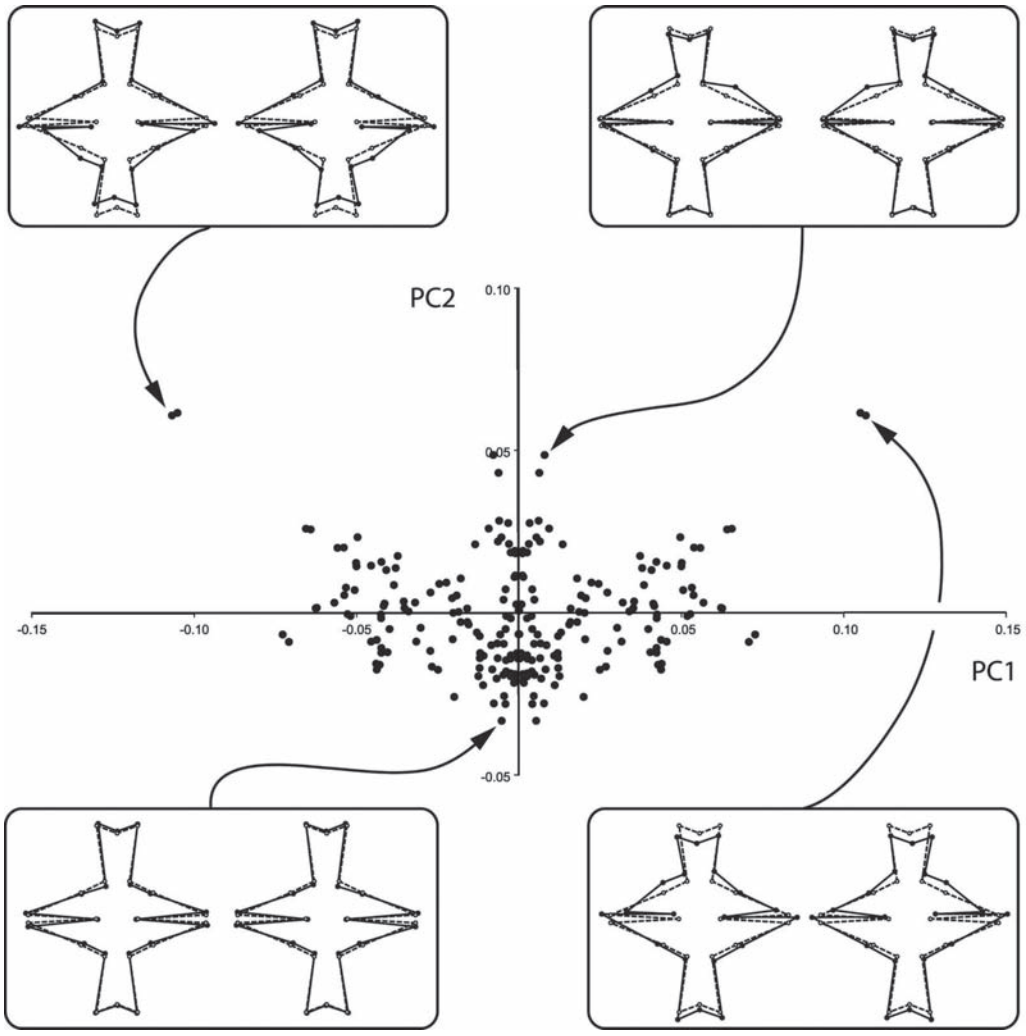

Fig. 4. Scatterplot of the scores for the first two PCs. The distribution of the scores is symmetric under reflection about the vertical axis. Boxes show the shapes that correspond to the data points at the respective arrowhead (solid circles and lines) in comparison to the overall mean shape (open circles and dashed lines). Each point in the plot corresponds to two copies of the same cell's landmark configuration, which differ by a reflection about the left-right axis (see text for details). Note that the boxes in the upper-left and lower-right corners of the diagram relate to the same cell; they differ by a reflection about the juvenile-adult axis, which is picked up by the PC1 but not the PC2 axis.

variate regression is statistically highly significant ($p < 0.0001$). This allometry can be visualized as the shape change that corresponds to an increase in centroid size by 20 micrometers (Fig. 6B). The allometric shape changes among cells correspond to landmark movements towards the isthmus at the bases of incisions between lateral lobules, polar lobes and lateral lobules, whereas the polar lobes become thinner and more elongate while moving away from the isthmus (Fig. 6B).

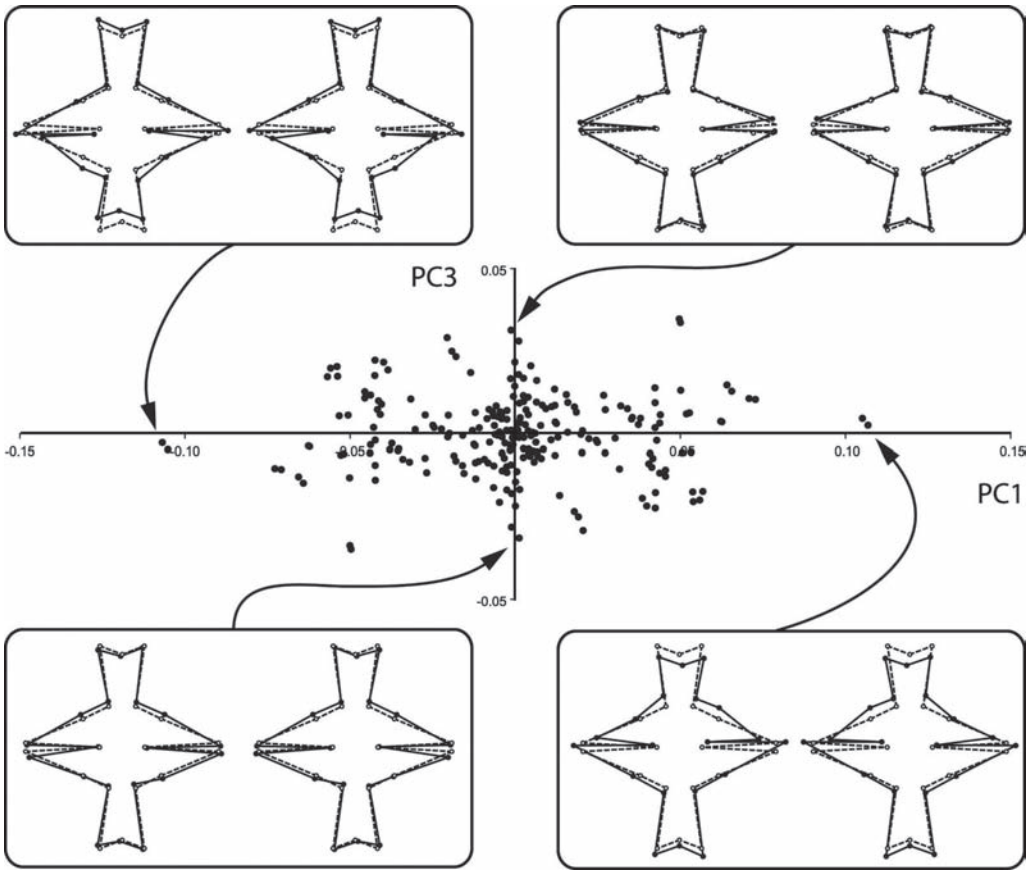

Fig. 5. Scatterplot of the scores for the first and third PCs. The distribution of the scores is symmetric under rotations of order 2 about the origin of the coordinate system. Boxes show the shapes that correspond to the data points at the respective arrowhead (solid circles and lines) in comparison to the overall mean shape (open circles and dashed lines). Each point in the plot corresponds to two copies of the same cell's landmark configuration, which differ by a reflection about the left-right axis (see text for details). The boxes in the upper-left and lower-right corners of the diagram as well as the boxes in the upper-right and lower-left corners relate to the same cell, respectively. The shapes in the two boxes of each pair differ by a reflection about the juvenile-adult axis, which is picked up by both the PC1 and the PC3 axis.

Discussion

In this paper, we have presented a new and general method for the analysis of shapes with complex symmetries and have applied it to study a sample of cells of *Micrasterias rotata*. The method can distinguish four components of variation according to their types of symmetry, which can be readily interpreted in relation to the biology of the cells under study (e. g., Meindl 1993). Our results suggest that differences between juvenile and adult semicells account for the bulk of morphological variation (62.3 % of the total variance), which is more than twice as much as the variation among cells (25.8 % of the total variance) and ten times more than the variation between left and right sides of cells (6.1 % of the total variance).

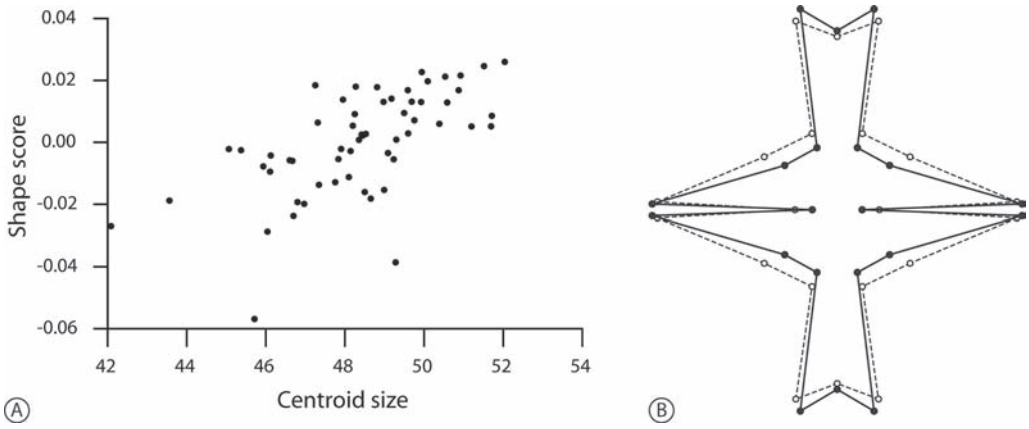

Fig. 6. Allometry of *Micrasterias rotata* cells for the completely symmetric component of shape variation. **A.** Shape scores obtained from the multivariate regression plotted against centroid size. **B.** Shape change associated with allometry. The differences between the symmetric consensus (open circles and the dotted lines) and the other configuration (solid circles and solid lines) represent the predicted shape change corresponding to an increase of centroid size of 20 micrometers.

The dominant role of the morphological differences between adult and juvenile semicells is underscored by the PC1, which accounts for nearly half of the shape variation on its own (Fig. 3A). Because the analysis considers the shape of the whole cells, variation in the relative sizes of the juvenile and adult semicells is a feature of overall shape, and is likely to be responsible for the dominance of the PC1. This PC represents the main shape change that is associated with cell growth and, due to the association between this shape change and the size differences between semicells, it can be interpreted as growth allometry within a single cell. The shape change associated with the PC1, in addition to the difference in relative size between the adult and juvenile semicells, is mainly an elongation of the polar lobe and a deepening of the incisions between the lobes in the adult semicell relative to those in the juvenile semicell, as well as a slight bending of the boundary between the two semicells toward the juvenile semicell (Fig. 3A). These patterns correspond to changes that can be observed directly during growth (Lacalli 1975).

The allometry of whole cells focuses on the slightly different question whether the shape of entire cells is associated with size. It can be studied with a multivariate regression of the symmetric component of shape variation on the centroid size of the whole cell. The associated shape change consists of a deepening of the incisions between the lobes, whereas the polar lobes become narrower and more elongate (Fig. 6B). This allometry is therefore quite similar to the contrast of shape changes between for the juvenile and adult semicells in the PC1 (Fig. 3A). The two types of allometry, which correspond to ontogenetic and static allometry, are therefore similar.

Similarly, Neustupa et al. (2008) used the same strain to study the allometry of adult semicells in the context of size differences between cells cultured at different temperatures. They analyzed allometry with a multivariate regression of the symmetric component of shape (in respect to reflection about the left-right axis) on centroid size and found allometric shape changes that were similar to those reported here. Neustupa et al. (2008) interpreted them in relation to a trade-off for stabilizing the surface-to-volume ratio of the cell. For any constant cell shape, the surface-to-volume ratio decreases with increasing cell size. For large cells, a shape change that deepens the incisions between lobes and extends the polar lobes will tend to increase the surface of the cells and thus increase their surface-to-volume ratio. Therefore, the deepening of the incisions

between lobes and the extension of the polar lobes may be an adaptive mechanism for stabilizing the surface-to-volume ratio of *Micrasterias rotata* semicells (Neustupa et al. 2008).

Other morphometric methods have also been applied to the study of complex symmetry in biological structures. For instance, Frey et al. (2007) suggested an approach for the analysis of rotational symmetry, but this framework is limited to the study of one landmark per repeated sector. Potapova & Hamilton (2007) published a method for shape analysis of diatoms that are symmetric relative to two perpendicular axes of symmetry. They used an original configuration of landmarks for the entire diatom and produced four transformed copies of it by applying reflections with the landmarks appropriately relabelled for each transformed copy. In fact, Potapova & Hamilton (2007) applied the four symmetry transformations that constitute the symmetry group. The original configuration represents the identity, there is the reflection about the vertical axis, the reflection about the horizontal axis that is equivalent to the reflection about the vertical axis combined with rotations by 180°, and two successive reflections about the two axes that correspond to rotations by 180° (Fig. 2). Their approach is identical to the method we used for *Micrasterias rotata* and is a special case of our general framework.

In this paper, we used a new comprehensive method for shape analysis of symmetry and allometry in *Micrasterias rotata* cells that exhibit complex symmetry. Our study revealed interesting patterns of symmetric and asymmetric shape variation related to size of the cells. In particular, we used the specific mode of growth in *Micrasterias rotata* to study two types of allometry: ontogenetic allometry within cells that indicate shape changes associated with growth between juvenile and adult semicells and static allometry among cells that reveal shape variation related to size for the whole cell. These approaches can be applied to the study of other interesting symmetries and growth in other algae (e. g., rotational symmetry in the arrangement of cells in many colonial algae such as *Volvox aureus*).

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