

Differential growth in *Tripneustes gratilla* (Echinoidea)

JACOB DAFNI & JONATHAN EREZ

Hebrew University of Jerusalem, H. Steinitz Marine Biology Laboratory, Eilat, Israel

1 ABSTRACT

A radiotracer technique, using ^{45}Ca , was applied to determine differential growth at the sutural margins of skeletal plates in the regular echinoid *Tripneustes cf. gratilla*. Directional growth in each interambulacral plate was determined by vector addition of the growth vectors that represent the increment in the four directions of the plate (adoral, aboral, interradial and adradial). Directional growth correlated with the spatial arrangement of the intestinal mesenterial threads that attach the intestine to the interambulacral plates. These threads may exert mechanical tensional forces on the plates and affect the differential growth pattern. This agrees with Raup's (1968) hypothesis that mechanical stresses influence the growth of the echinoid skeletal plates and determine the overall test shape.

2 INTRODUCTION

Echinoid skeletal plates grow in a differential pattern which determines the shape of the test during ontogenetic development (Märkel, 1975) and influences phylogenetic trends during evolution (Seilacher, 1979). An interaction exists between the growth of skeletal parts and the changes that take place in the inner dermal capsule and other soft tissues, collectively termed the "functional matrix" (Moss & Meehan, 1968). Raup (1968) observed similarities between the shapes of echinoid plates and other "close-packing" structures and suggested that mechanical forces may take part in the regulation of echinoid growth. No detailed description was given to show

exactly how such regulation occurs and what tissues are involved. The intestine supporting mesenteries may influence the echinoid growth and the incidence of skeletal deformations.

This paper correlates patterns of differential growth with the spatial arrangement of the mesenteries and tube feet and discusses their possible biomechanical function.

3 MATERIAL AND METHODS

Live *Tripneustes cf. gratilla*, a local variant of the Indo-Pacific *T. gratilla*, were collected in the northern Gulf of Eilat, and held in filtered seawater with $^{45}\text{CaCl}_2$ (activity of 15 $\mu\text{Ci/ml}$). The glass containers were aerated and maintained at room temperature of $\sim 22^\circ\text{C}$. After 8 hrs of daylight incubation the animals were washed three times with unlabelled seawater, killed by exposure to air, and immersed for 20 hours in 5.5% NaOCl solution. Throughout the experiment a pH above 8 was maintained to prevent loss of ^{45}Ca from the skeleton. The test was oven dried (60°C) after the final washing.

For the determination of Ca uptake by individual plates, each interambulacral (IA) plate in one complete row was weighed and its ^{45}Ca activity determined by liquid scintillation, using Packard tri-carb 3255 spectrometer. Two ml Instagel scintillation cocktail (Packard) in 5 ml polypropylene minivials were used to count the samples. Preliminary results showed that it is not necessary to dissolve the skeletal material prior to immersion in Instagel. The counting efficiency was 85% and the results were corrected accordingly. Differential growth was determined as follows: slices of ca. 300 μm width were

cut with a dissecting knife from the sutural margins of IA plates (interradial, adradial, adoral and adapical) (Fig. 2A). The median part of the plate which remained after removal of the sutural margins was also sampled. There were five components for each plate. Each component sample was dried, weighed with a Cahn 25 electrobalance to the nearest μg , and its ^{45}Ca activity counted. Since ambulacral plates of *Tripneustes* are very narrow, a somewhat different treatment was applied: the perradial triangle from each plate and the adradial edge were sampled. The remaining part was cut into two, the adapical and adoral halves. Fractions from five consecutive ambulacral plates were pooled into one sample and counted. Growth was calculated by dividing the ^{45}Ca uptake (counts) by the specific activity of the seawater and the weight of the sample analyzed.

The precision of the analytical technique was determined by replicates of the same ground calcareous samples. The standard deviation (2σ) is 10-30% of the reported values. The reproducibility of the calcification rates between homologous plates of different columns, determined by comparing three IA plate columns of the same individual, was better than 20% (2σ). Zero-time control was determined by exposing living animals for 0.5 min to the radioactive seawater. Animals killed by immersion in liquid nitrogen and incubated for 24 hr in radioactive seawater served as dead control. The Ca uptake by 0-time control and dead control, compared with live sea urchins, were 0.3% and 4% respectively.

4 RESULTS

For a medium sized (49 mm) sea urchin the total calcium uptake was lowest for IA plates at the peristomial margin and near the apex, and the highest uptake was observed in plates above the ambitus. Relative growth (mg Ca added per mg skeletal CaCO_3 per hr) was highest for plates of the apical region. Differential growth (i.e. the different growth rates shown at different suture margins) for IA plates follows a definite pattern (Fig. 1): maximum growth occurred in the interradian direction (the longitudinal middle suture of the interambulacrum) in plates of the superambital region. The interradian growth slowed considerably below the ambitus. Growth rate in the adradial direction was lower, but it persisted even in the lowermost plates, at the peristome

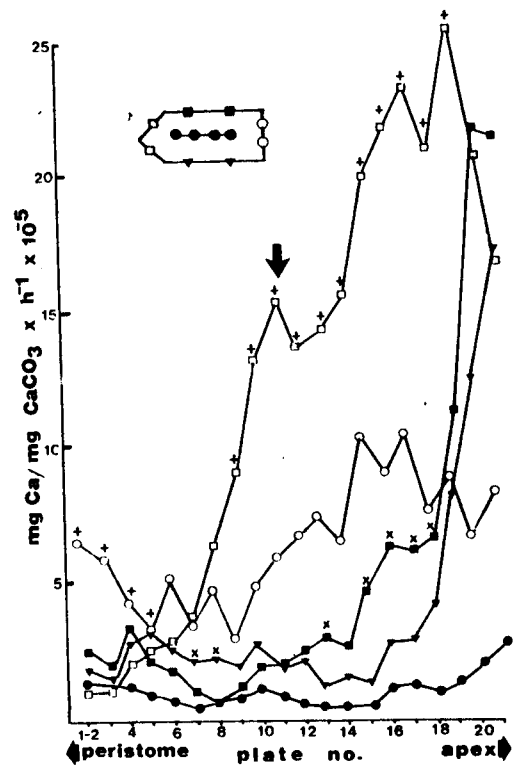


Fig. 1. Growth, expressed as Ca uptake rates, at the median part (\bullet) and various sutural margins (\circ - adradial, \square - interradian, \triangle - adoral and \blacksquare , adapical directions) of interambulacral plates of a 49 mm *T. cf. gratilla*. Plate sequence (horizontal axis) in the IA column starts at the peristome and rises toward the apex. Each point represents the means of 4 replicates. + and x indicate significant differences in growth rates between sutures of adverse directions of the same plate, horizontal and vertical axes, at $p < 0.05$ (T-test, pairs). Arrow points to the position of the ambitus.

margin. The growth rate of the median part of the plates, which represents the calcification of the inner and outer surfaces, and thickening of the individual skeletal microelements (trabeculae) remained low for all plates. The meridional directed growth also showed typical features; the growth at the apical

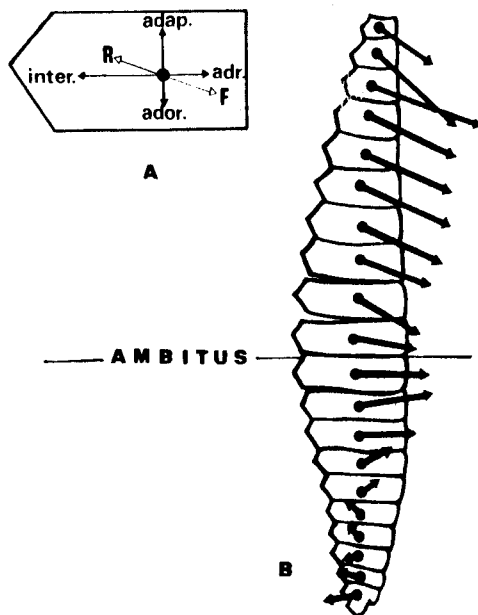


Fig. 2. (A) Vector addition of growth rates at the different directions in an IA plate. R, resultant growth vector, F - inferred tensional force vector. (B) Map of F vectors for plates along the IA column.

direction (adapical sutures) exceeded the adoral growth in the superambital plates while in the subambital plates an opposite trend was shown. The growth rates in adverse directions of each plate (e.g. between adoral and adapical growth) were significant, (paired T-tests, $p < 0.05$) for most plates (Fig. 1).

An assumption was made that the plates behave like rigid objects with a certain amount of freedom to move relatively to one another (Thompson, 1942). By representing the growth at the various directions of the plate by vectors and by using vector addition, R (the resultant growth vector) was calculated (Fig. 2A). This calculation assumes that growth of a plate potentially occurs in all directions. Adding the growth vectors eliminates the uniform growth components and the resultant vector represents only the directional growth component. Such directional growth may result from relative tension exerted on the plate due to a pulling force of a proportional intensity, but inversely directed (F).

Repeating this application for all the test plates, a map of the hypothetical tensional forces vectors is reconstructed (Fig. 2B) which derives from the differential growth data (Fig. 1).

The distribution pattern of the F vectors for the IA plates row (Fig. 2B) correlates with the spatial arrangement of the mesenterial festoons that attach the digestive system to the inner surface of the test (Fig. 3). According to Hyman's (1955) and our observations, the small intestine is fixed to the test by threads that split from the mesenteries and attach to the IA plates. The uppermost attachment of this intestine is halfway between the ambitus and the apex (Fig. 3A), spanning across the ambulacral plates without being attached to them. In its lowest parts it is attached to IA plates on both sides of the interradiial suture. The large intestine, or second loop, overlaps with the small intestine mesenterial attachment. The only apparent difference in attachment is that it reaches the apex and hangs from the uppermost IA plates (Fig. 3B). The attachment of the intestine to the inner test wall probably involves mechanical forces that are applied through the mesenterial threads, whose general arrangement (Fig. 3) follows the pattern indicated by the hypothetical tension vectors (Fig. 2B).

An analysis of the differential growth of the ambulacral plates is shown in Fig. 4. The maximal growth increment was shown at the adradial suture (joining the IA plates) of all the ambulacral plates. In the meridional direction the adapical growth exceeded the adoral growth in all plates along the ambulacral column. This is a quite different pattern than the growth pattern of the IA column. The calculated F vectors should be directed towards the mid suture of the ambulacrum (perradial) with a vertical downwards component. The mechanical forces that are involved in such directed growth are probably the downwards pull of the tube feet and forces applied by other components of the ambulacral system. The forces that are exerted by the lantern retractor muscles, may also affect the ambulacral plates growth and contribute to the differential growth pattern.

5 DISCUSSION

Differential growth in echinoids was described from natural growth lines (Deutler, 1926) and from the application of tetra-

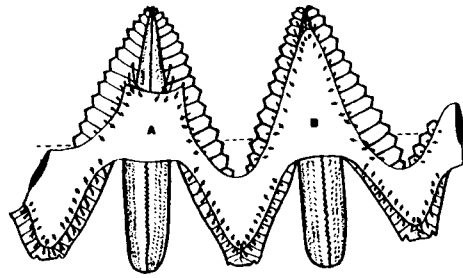


Fig. 3. Schematic representation of two *Tripneustes radii*, consisting of interambulacral (large plates) and ambulacral (small plates) columns, the arrangement of the small (A) and the large intestine (B) and their attachment to the test plates by mesenterial threads. Arrows show the possible directional tension caused by the individual threads. Dashes indicate the position of the ambitus.

cycline (Kobayashi and Taki, 1969). Both techniques need a considerable length of time to allow proper measurement and the latter fails under tropical conditions (Barnes, 1971; Ebert, 1980). The data from ^{45}Ca labelling presented here agree well with the differential growth patterns described by Märkel (1975) in *Paracentrotus lividus* (his Fig. 6). Shorter experimental time and greater flexibility in experimental design are the major advantages of the ^{45}Ca method (Märkel's data, for comparison, represent the growth increment of 10 mths).

Thompson (1942) wrote, "The rigidity of the shell is more apparent than real, for the entire structure is, in a sluggish way, plastic...The entire shell grows by increments to each and all multitudinal elements, whose individual growth involves a certain amount of freedom to move relatively to one another". He also believed that the form of the sea urchin is subject to an "equilibrium of forces". Raup (1968) concluded his detailed study of the growth gradient along the plate columns with the suggestion that the growth gradient is a result of the change of stresses that inhibit growth.

If these ideas are acceptable, there is a need to find the constructional elements that produce the forces that encourage or inhibit the plates growth in the various directions, thereby affecting the test form. Dafni (1980) suggested that the

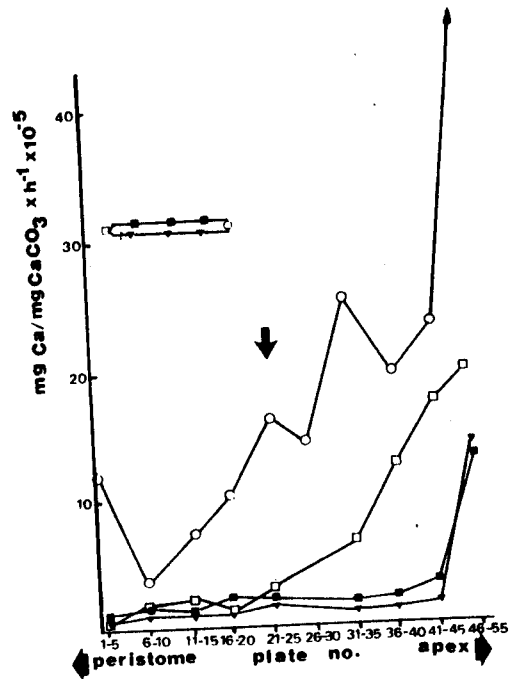


Fig. 4. Growth rate pattern in the ambulacral plate column. Symbols are the same as for Fig. 1. Each point represents five consecutive plates, pooled into one sample.

arrangement of various elastic and contractile tissue elements, such as tube feet, lantern retractor muscles and probably also the collagenous sutural fibers, exert mechanical forces that may control the growth pattern of the plates. The ambulacral tube feet are the most prominent force producing element, contributing to the ability of sea urchins to withstand wave action (Sharp & Gray, 1962). They emerge from the ambulacral plates and apply considerable mechanical force towards the substrate. Thompson (1942) postulated that these forces tend to depress the test, causing the "flattened cake" appearance of many regular sea urchins.

Skeletal deformation occurs in *T. cf. gratilla* populations in polluted localities in the Red Sea (Dafni, 1980). The deformed urchins show either a sunken apex or extreme bulging of the aboral half. In the former, regular depressions occurred in the apical end of each ambulacrum. "Pinching", or narrowing of the ambulacra at the superambital region, characterized the latter abnormality. Since these areas of the test coincide with the upper attachment points of the inner mesenteries,

the mechanical effect of the mesenterial pull, coupled with softening of the test, probably produced these deformities. Hamann's (1887) description of a cross section of a mesenterium as consisting of outer epithelial cell layers and filled with connective tissues and muscle fibers may support these hypotheses.

A close packing pattern shown by echinoid plates (Raup, 1968) indicates that confinement between the plates may limit their peripheral growth. Collagenous fibers, which connect the plates together across the sutures, contribute to the test rigidity through their mechanical properties. Directional growth of the collagenous fibers or other components of the organic matrix may also cause differential sutural calcification (Roux, 1975). However, the tensional forces that are produced by the elastic and contractile tissue elements, mesenteries and tube feet at definite directions add a mechanical constraint to the growing test, tending to modify its shape.

It is still unclear whether this effect is purely physical, or of a thermodynamic or kinetic nature which affects the CaCO_3 solubility product, or if more complex physiological and biochemical processes are involved.

6 ACKNOWLEDGEMENTS

We gratefully acknowledge the interest and encouragement provided by Prof. F.D. Por, and thank the H. Steinitz Marine Laboratory staff for their assistance. We are also grateful to Mrs. A. Goodman for correcting and typing the manuscript.

7 REFERENCES

- Barnes, D.J. 1971, A study of growth structure and form in modern coral skeletons, Ph.D. Thesis, University of Newcastle upon Tyne.
- Dafni, J. 1980, Abnormal growth patterns in the sea urchin *Tripneustes cf. gratilla* (L.) under pollution (Echinodermata, Echinoidea). *J. exp. mar. Biol. Ecol.* 47: 259-279.
- Deutler, F. 1926, Über das Wachstum des Seeigelskeletts. *Zool. Jb. (Anat.)* 48: 119-200.
- Ebert, T.A. 1980, Relative growth of sea urchin jaw: an example of plastic resource allocation. *Bull. Mar. Sci.* 30: 467-474.
- Hamann, O. 1887, Beiträge zur Histologie der Echinodermen. Vol. 3, Jena, Gustave

- Fischer Verlag.
- Hyman, L.H. 1955, *The invertebrates*. Vol. IV. Echinodermata. New York, McGraw-Hill.
- Kobayashi, S. & J. Taki 1969, Calcification in sea urchins. I. A tetracycline investigation of growth of the mature test in *Strongylocentrotus intermedius*. *Calcif. Tissue Res.* 4: 210-223.
- Märkel, K. 1975, Wachstum des Coronarskeletes von *Paracentrotus lividus* Lmk. (Echinodermata; Echinoidea). *Zoomorphologie* 82: 259-280.
- Moss, M.L. & M.M. Meehan 1968, Growth of the echinoid test. *Acta anat.* 69: 409-444.
- Raup, D.M. 1968, Theoretical morphology of echinoid growth. *J. Paleontol.* 42 (suppl. to no. 5): 50-63.
- Roux, M. 1975, Microstructural analysis of the crinoid stem. *Univ. Kans. Paleontol. Contrib.* 75.
- Seilacher, A. 1979, Constructional morphology of sand dollars. *Paleobiology* 5: 191-221.
- Sharp, D.T. & I.E. Gray 1962, Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. *Ecology* 43: 309-313.
- Thompson, D.W. 1942, *On growth and form*, 2nd edition. London, Cambridge University Press.