veloped only in cells that had lost normal control of growth (*14*). It is possible that in our experiments the activated macrophage is reacting to C-type virus. This explanation for the reactivity of activated macrophages against mouse cell lines is probably insufficient, because the spontaneous development by cells of abnormal growth properties in vitro is not always associated with the release of C-type virus (*14*).

We showed earlier that mice having a population of activated macrophages with increased in vitro cytotoxic capability (*8*, *9*) also have increased resistance to autochthonous and transplanted tumors (*7*, *10*, *12*, *15*). These results, coupled to those reported here for the in vitro system, provide further evidence that the activated macrophage may have a role in the control of newly emergent cells with abnormal growth properties in vivo (*8*, *9*).

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References and Notes

1. We define a cell strain and a cell line according to the criteria of Hayflick and Moorhead (*2*), with the added qualification that we are referring in this report to a specific type of cell line, one that has lost contact inhibition of cell division. By these criteria, all cell strains have limited capacity for multiplication and normal growth properties, while cell lines have an unlimited capacity for multiplication and in addition may exhibit one or more abnormalities of in vitro cell growth, which include density inhibition of growth (contact inhibition of cell division) [M. G. P. Stoker and H. Rubin, *Nature* **215**, 171 (1967)].
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Genetic Meaning of Zooecial Chamber Shapes in Fossil Bryozoans: Fourier Analysis

Abstract. *Fourier harmonic amplitudes quantitatively characterize chamber shapes of fossil tubular bryozoans. The odd-numbered harmonics, particularly the seventh, carry evolutionary information. The phenotypically plastic second and sixth harmonics measure zooecial orientation and packing, respectively. As a measure of crowding, the sixth harmonic reflects mechanistic growth response to paleoenvironmental conditions.*

Fourier analysis quantifies shape differences among zooecial chambers of fossil bryozoans. Genetics, growth, orientation, and packing control shape components. The sixth harmonic reflects packing and gross colony form; the second reflects orientation and, in ramose forms, ontogeny. Distance from a monticular center controls the fourth, fifth, and sixth harmonics. The other harmonics vary little within a colony, but widely among evolving taxa.

Ehrlich and Weinberg (*1*) have shown that two-dimensional closed shapes can be characterized to any desired degree of precision by using the harmonic coefficients of the Fourier series as shape descriptors (*2*). Younker (*3*) and Kaesler and Waters (*4*) have demonstrated the utility of Fourier harmonics in the study of ostracode carapaces. Each harmonic makes an independent contribution to the total shape: The first describes the contribution of an offset circle, the second of a figure eight, the third of a trefoil, and the fourth of a quatrefoil. The *n*th harmonic describes the contribution made to a given shape by a "clover" with *n* leaves. All equilateral polygons with *n* sides yield a high amplitude in the *n*th harmonic and in higher harmonics that are multiples of *n*.

Harmonic spectra from tangential sections of a large hemispherical colony of *Amplexopora filiasa* (Fig. 1) show considerable shape variation among genetically identical zooids (*5*). The chambers fall into three shape families: monticular zooecia (megazooecia), zooecia in the aureole surrounding each monticule (*6*), and zooecia of the intermonticular areas. Megazooecia are generally quadrate, and so have a high fourth harmonic. Zooecia in the monticular aureole are generally pentagonal, displaying a high fifth harmonic. Intermonticular zooecia, however, most commonly hexagonal, have a high sixth harmonic (Fig. 2A). The second harmonic also varies within a colony. Megazooecia and aureole zooecia are both more elongate than intermonticular zooecia, and so have higher second harmonics. Their elongation results from the elevation of the monticule above the colony surface, forming a wartlike protuberance in which the tubes are inclined to the colony surface and therefore appear elongate in section. The second harmonic measures the deviation from the perpendicular. In variously oriented cross sections of the same zooecial prism, only the second harmonic changes; the others remain constant.

When most densely packed, circular cylinders pressed together will become hexagonal prisms (*7*), and their amplitude spectra will display prominent sixth harmonics. Intracolonial spectra (Fig. 2A) suggest that packing is lowest in the monticules and increases toward the intermonticular regions; the spectra show a radial packing gradient corresponding to a decrease in zooecial diameter. This gradient, and the space-filling structures (denser wall material and "mesopores") in the monticular centers (Fig. 1), suggest that monticules represent the budding centers of the colony, and that the packing gradient indicates successive stages of zooecial ontogeny. As the cylinders extend themselves distally, new zooids are budded interstitially in the monticules. As more zooids are budded, they are pushed away from the monticule into the monticular aureole, and finally into the densely packed intermonticular area. In ontogeny, the zooids decrease in size, change orientation, and develop additional sides until their cross sections ultimately become equilateral hexagons.

The mean harmonics of different colonies illustrate the variation among different taxa and growth forms (Fig. 2B). The amplitude spectra of three

genera (represented by *Hallopora nodulosa, Amplexopora septosa,* and *Balticoporella whitfieldi*) suggest that the means display enough differences to distinguish these taxa from each other statistically, even without partitioning the Fourier variables. This is not unexpected, inasmuch as the harmonics exactly described the skeletal phenetics, on which the taxonomic concepts were based. The genetic makeup of these organisms, however, does not restrain some of the phenetic shape components (Fig. 2B) within any observable limits, but allows changes to be imposed by external or internal stresses. Zooecial chambers in *H. nodulosa* are more elongate than in the other two zoaria, resulting in a higher second harmonic. As in the monticules of *A. filiasa*, the zooecia meet the colony surface obliquely. In ramose trepostomes, zooecia become less oblique as the zooids grow older. Thus, the second harmonic reflects, in part, the ontogenetic stage of the zooids at the level sectioned.

The sixth harmonic reflects the colony's packing, growth form, and number of mesopores. The *Hallopora* colony (Fig. 2B) is a thin branching form, whereas the other two specimens are robust or thick branching forms. Both of the latter lack mesopores, whereas *Hallopora* has abundant mesopores located interstitially among the zooecia. Many workers have reported the control of gross colony morphology by water agitation, which correlates with the percentage of encrusting or hemispherical forms (*8*). Because bryozoans filter suspended food from the water, such control could be in part nutritional: Being closer together in a colony, as in a hemispherical form, diminishes the volume of water available to each zooid for feeding and dissipating metabolic wastes. Shallow agitated waters contain abundant planktonic food, and a greater volume of water moves past each feeding zooid. Thus, the zooids do not need to be far apart to obtain adequate food and remove wastes, and the colony need devote less energy to skeletal construction because close packing minimizes the volume of calcium carbonate needed to construct the zoarium. In quiet water the zooids would obtain more food and dissipate wastes more effectively if they were more widely separated, although the colony would have to build space-filling structures to position each zooid within a greater water volume and strengthen the thin branching colony. Water agitation, therefore,

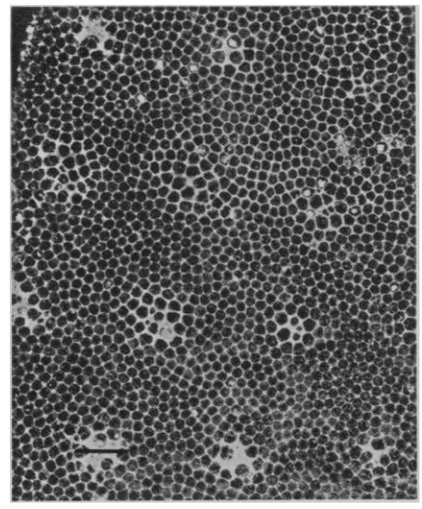

Fig. 1. Photomicrograph of a tangential section of *Amplexopora filiasa* (D'Orbigny). The specimen is in the collection of Michigan State University (specimen number MSU 1001-204). The dark line at the lower left represents 1 mm.

could control zooidal packing, with consequent shape modifications of the living chambers. Hence, hemispherical zoaria would have higher sixth harmonics than thin ramose zoaria (Fig. 2, A and B); the robust or thick branching forms would have intermediate values (Fig. 2B). The second and sixth harmonics, therefore, vary considerably within a clone and, in different clones, change as a result of mechanistic growth response to environmental conditions.

To test the systematic value of each harmonic, we digitized zooecial shapes from Devonian trepostomes illustrated by Boardman (*9*). To carry evolutionary information, a harmonic should vary little within a colony, but greatly among evolving taxa, as among the species of a genus. To determine intraclonal variability, we calculated the coefficient of variability, CV ($CV = 100$ S.D./$\bar{x}$, where S.D. is the standard deviation and $\bar{x}$ is the mean), for each of seven taxonomically different colonies for the first ten harmonics; we used between 8 and 11 shapes each (*10*). Intrageneric CV's were calculated for 23 species belonging to four genera by using 210 digitized shapes (*11*). The mean intrageneric CV of each harmonic was divided by the mean intraclonal CV (Fig. 2C). Values greater than 1.0 indicate greater variance among evolving taxa than within a clone. Values less than 1.0 indicate great phenotypic plasticity that does not reflect evolutionary change. Only the second and sixth harmonics fall below 1.0, which confirms their control by nongenetic factors. The odd-numbered harmonics, especially the seventh, carry most of the evolutionary information.

Unfortunately, the second and sixth harmonics make major contributions to the visual impact of a given shape. Consequently, the degree of ellipticity and hexagonality have figured as major biocharacters in bryozoan systematics. The sixth might have adaptive importance, because more densely packed colonies feed, dissipate waste, and construct their skeletons more easily, which prob-

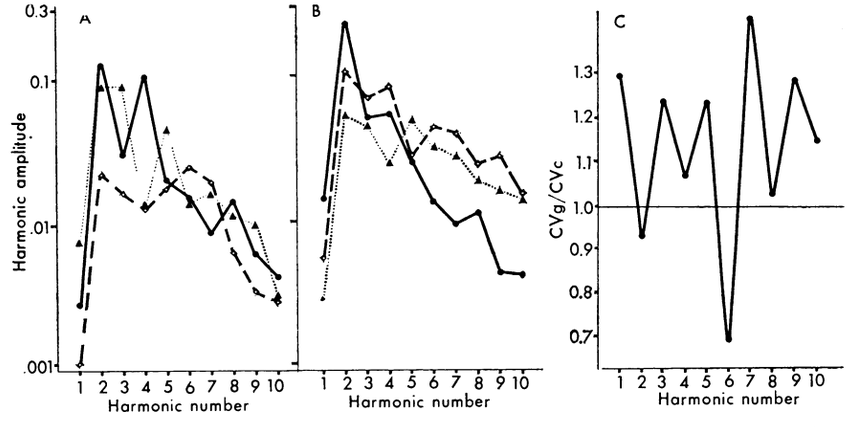

Fig. 2. (A) Harmonic amplitude for each harmonic number of zooecial shapes from *Amplexopora filiasa*. (Solid line) Quadrate shape in the monticule; (dotted line) pentagonal shape from the aureole; (dashed line) hexagonal shape from the intermonticular area. (B) Harmonic amplitude for each harmonic number of mean zooecial shapes from colonies of *Hallopora nodulosa* (Nicholson) (solid line); *Amplexopora septosa* (Ulrich) (dashed line); and *Balticoporella whitfieldi* (James) (dotted line). The specimens are in the collection of Indiana University (specimen numbers IU 8975-41024, 8973-11001, and 8975-38001, respectively). (C) Ratio of mean intrageneric coefficient of variability (CV_g) to mean intraclonal coefficient of variability (CV_c) for each harmonic number of zooecial shapes from Middle Devonian trepostomes.

ably results in greater reproductive success. However, the wide variance of the sixth within a clone suggests that different packing arrangements and growth forms of tubular bryozoan colonies result from nongenetic causes.

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12. We thank R. Ehrlich, J. Holman, and R. Taggart for their help.

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Adenosine 3′,5′-Monophosphate and N^6-2′-O-Dibutyryl-Adenosine 3′,5′-Monophosphate Transport in Cells

Abstract. *Incubation of Strain L cells in 0.15 millimolar adenosine 3′,5′-monophosphate (cyclic AMP) increase the intracellular level of cyclic AMP more than does incubation in dibutyryl cyclic AMP. The intracellular increase of cyclic AMP is greater with Strain L than with WI-38 cells. Similarly, cell division of Strain L cells is inhibited more by cyclic AMP. The experiments do not support the belief that dibutyryl cyclic AMP is transported better than cyclic AMP.*

In spite of numerous articles which state that N^6-2′-*O*-dibutyryl-adenosine 3′,5′-monophosphate is transported more readily than adenosine 3,′5′-monophosphate (cyclic AMP), there is as yet no direct evidence that supports this conclusion (*1*). The investigations usually cited as evidence for this statement suggest rather than establish this point (*2*). The situation is aptly described by Robison, Butcher, and Sutherland: "There is no direct evidence that the derivatives penetrate cell membranes more rapidly than cyclic AMP, although this may occur and may in some cases be the explanation for their greater potency" (*3*).

The purpose of this investigation was to obtain evidence regarding the transport of these two biologically important compounds in cell cultures.

Strain L (NCTC, clone 929) and WI-38 cells (38th or 39th passage) were used. The cells were determined to be mycoplasma-free by the method of Hayflick (*4*). The cell cultures were maintained in Eagle's minimal essential medium with Hanks' salts. Glutamine (29.2 mg/liter) was added to the medium and the *p*H was adjusted to 7.6 with 0.89*M* bicarbonate. The medium was supplemented with fetal calf serum (10 percent), neomycin (50 mg/liter), and amphotericin B (2 mg/liter).

Inhibition studies of cell cultures by cyclic AMP and dibutyryl cyclic AMP were done in Leighton tubes seeded with 25,000 cells per milliliter, total volume 2 ml. On the second and fourth days of incubation the cells were removed with 0.25 percent trypsin and counted in a Coulter counter.

Adenosine 3′,5′-monophosphate acid (Sigma, lot Nos. 90C-2080, 81C-0220, and 78B-7240), N^6-2′-*O*-dibutyryl-adenosine 3′,5′-monophosphate, monosodium salt (Sigma, lot Nos. 90C-7020 and 91C-7200), and butyric acid were dissolved directly in the culture medium and filtered through a prewashed Millipore filter (0.22 μm size). The butyric acid was redistilled, and the concentration was determined by titration. The medium containing cyclic AMP and dibutyryl cyclic AMP was used the day of preparation because of the phosphodiesterase activity in fetal calf serum.

Cells for the cyclic AMP assay were grown in T-60 flasks seeded with 50,000 cells per milliliter, total volume 20 ml. After incubation, the medium was removed and the cell layer was rinsed six times with ice cold, 0.85 percent sodium chloride. Removal of all extracellular cyclic AMP was indicated by no further decrease in cyclic AMP with additional washes. Along with each experimental T-60 flask, a control flask with no cells but containing medium and cyclic AMP was employed to determine the effectiveness of the washing procedure in removing all cyclic AMP from the glass. Because of the known binding of cyclic AMP to proteins, an additional flask of cells was used for determining the amount of protein-bound cyclic AMP. The cells were frozen and thawed repeatedly to disrupt the cell membranes, and the disrupted cells were washed six times with cold saline. The disrupted cells bound less than 1 pmole of cyclic AMP per million cells. For estimation of percent recovery, 5 ml of 0.5*N* perchloric acid in 25 percent ethanol containing tracer cyclic AMP [0.009 μc of [H^3]cyclic AMP (Amersham/Searle) per milliliter, 24.1 c/mmole] was added to the cell layer. The cells were removed from the glass by gentle scraping and centrifuged at 2000 rev/min for 15 minutes to remove the cell protein precipitate. After neutralization with KOH, the potassium perchlorate was removed by centrifugation, and the supernatant was

Table 1. Intracellular (In.) and extracellular (Ex.) concentrations of cyclic AMP and dibutyryl cyclic AMP in Strain L cells and in WI-38 cells. Values are millimolar and the average of three experiments. The molarity was calculated as millimoles of cyclic AMP per liter of packed cell volume. The cell volume was determined by microhematocrit.

Incubation time (days)	Dibutyryl cyclic AMP (m*M*)			Cyclic AMP (m*M*)		
	In.	Ex.	In./Ex.	In.	Ex.	In./Ex.
			Strain L cells			
0		0.15			0.15	
1	0.07	.14	0.5	1.01	.07	14.4
2	.04	.12	.3	0.14	.03	4.7
3	.01	.11	.1	.09	.02	4.5
4	.005	.09	.06	.10	.02	5.0
			WI-38 cells			
0		0.15			0.15	
1	0.10	.14	0.7	0.10	.03	3.3
2	.17	.10	1.7	.15	.002	75.0
3	.06	.08	0.8	.02	.0008	25.0
4	.05	.07	.07	.02		