

NOTE

A new theory to explain the origin of growth lines in sea urchin spines

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ABSTRACT: A new theory is proposed to explain the origin of some growth lines in echinoid spines. It is proposed that lines form as part of a growth event initiated when a usually non-growing spine no longer fits the growing tubercle on which it sits. Growth lines also can form in response to damage but neither cause of growth lines is periodic and hence lines cannot be used to determine age.

There have been 2 theories to explain the origin and significance of growth lines in sea urchin spines. These are: (1) growth lines are periodic and so can be used to estimate the age of an individual (Carpenter 1870, Deutler 1926, Moore 1966, Weber 1969, Dotan & Fishelson 1985); and (2) growth lines are the result of repair following trauma, which can be breakage or other damage (Ebert 1967, 1968).

The purpose of this note is to suggest a third hypothesis for growth line formation; namely, (3) some growth lines form during non-periodic growth episodes when a discontinuously growing spine no longer fits its continuously growing tubercle.

I have argued previously (Ebert 1985) that the hypothesis of periodic development can be eliminated. Growth lines in spines cannot be periodic because the relation between test length and number of rings in spines is linear. This was shown (Ebert 1985) by using data from *Heliocidaris erythrogramma* (Moore 1966), *Strongylocentrotus purpuratus* (Ebert 1967, 1968, 1985), and *Heterocentrotus mammillatus* (Ebert 1985). Weber (1969) presented data for *Heterocentrotus trigonarius* in which the relation of diameter vs number of rings is slightly curved upward. On the other hand, McRae (1959) found no correlation between size and number of growth lines in spines of *Evechinus chloroticus*; small specimens had the same number of rings as large individuals. Her illustrations, however, look like totally regenerated spines so a linear relation would not be expected.

If growth lines were periodic then the relation of test

size vs number of lines would have the shape of a growth curve; it would be a decaying exponential of the general Brody-Bertalanffy type or a Richards function with a negative shape parameter (cf. Ebert 1980). A plot of size at time $t+1$ vs size at time t (Walford 1946, Ebert 1980) should be a curve or straight line that approaches the 45° line of zero growth.

All sea urchins that I have studied (Ebert 1967, 1968, 1977, 1982) or reviewed (Ebert 1975) show decaying exponential growth curves. Dotan & Fishelson (1985), however, report linear growth for *Heterocentrotus mammillatus* in the Gulf of Eilat north of Sharm al Sheikh. Given the usual growth pattern of echinoids, I find their linear relation to be very unlikely. A linear growth curve for test length would require exponentially increasing growth in mass.

Because the relation between test size and age does not have the same shape as the relation between size and number of growth lines in spines (except as reported by Dotan & Fishelson), I reject the hypothesis that growth rings are periodic. They cannot be annual (Dotan & Fishelson 1985) or of some shorter period, such as daily or monthly, as suggested by Weber (1969).

I have rejected the periodic hypothesis and have favored the trauma hypothesis for growth line formation (Ebert 1985), which I have considered to be sufficient to explain the phenomenon. Trauma causes lines to form in *Strongylocentrotus purpuratus* (Ebert 1967, Heatfield 1971) and in *Heterocentrotus mammillatus* (Dotan & Fishelson 1985, Ebert 1985). There are, however, several problems with the trauma theory:

(1) Regeneration lines formed following breaks in *Strongylocentrotus purpuratus* spines may extend all the way to the milled ring, just above the base of the spine (Ebert 1967, Heatfield 1971). In *Heterocentrotus mammillatus*, regeneration lines tend not to develop proximally to reach the milled ring. They may extend

only a few millimeters proximally (Dotan & Fishelson 1985, Ebert 1985) so although trauma results in lines, it seems insufficient as the cause for major lines that extend from the milled ring to the spine tip.

(2) Lines that develop from breaks or other trauma such as holes drilled into the spine develop proximally (Heatfield 1971, Dotan & Fishelson 1985) whereas growth line formation also has been observed starting at the milled ring and spreading distally (Weber 1969, Heatfield 1971, Dotan & Fishelson 1985).

(3) Oral spines in *Heterocentrotus mammillatus* have more growth lines than ambital or aboral spines (Dotan & Fishelson 1985). Oral spines are the oldest on a sea urchin and, because of their position, would be protected from abrasion and, hence, would be expected to have fewer rather than more lines.

(4) Tagging *Heterocentrotus mammillatus* spines with tetracycline usually failed to mark the growing surface of spines (Ebert 1985).

(5) Abrading *Heterocentrotus mammillatus* spines with sandpaper failed to initiate line formation (Ebert unpubl.).

The following theory is designed to explain results of published studies and solve the 5 problems listed above. Trauma has been shown to cause lines in one sea urchin with relatively small spines, *Strongylocentrotus purpuratus* (Ebert 1967, Heatfield 1971), and it is reasonable to interpret the lines in spines of *Echinometra mathaei* illustrated by Borig (1933) as damage repair lines. The following theory is not intended to replace the trauma theory entirely but to explain lines that cannot be explained in terms of damage repair. Parts of the following theory have been tested, but no comprehensive set of interlocking experiments or observations have been made that could falsify it.

Allometry theory of growth line formation. The size of a sea urchin spine is a function of species, individual size, and position on the test and there is communication between the test and spines to maintain the correct allometric relations during growth. Although spines of most sea urchins have an epidermis, they do not grow continuously to maintain a size appropriate for their position. The test, on the other hand, is able to grow continuously. Tubercles on which spines articulate, because they are structures on the test, also grow continuously. The linking of a continuously growing test with non-growing spines causes a tension to develop between a spine base and the tubercle or mammelon on which it sits. Eventually the tension exceeds some threshold condition and a growth event is triggered, which causes a growth line to form. Growth episodes are size rather than time dependent, which is the meaning of the linear relation between test diameter and number of growth lines. Several

lines could be added to a spine during the first year of life but in large individuals many years could separate growth episodes of a spine.

The theory explains the 5 problems posed above:

(1 & 2) In small spines, such as those of *Strongylocentrotus purpuratus*, breaks are sufficient to cause regeneration to extend proximally to the milled ring and give rise to a new cycle. In *Heterocentrotus mammillatus*, a new growth episode is required to initiate a new cycle, which starts at the milled ring and extends proximally. The observation of Heatfield (1971) of growth line formation in a *S. purpuratus* starting at the milled ring would also be of this type.

(3) Oral spines are older than aboral spines and are more protected from physical damage. For the allometry theory to explain greater numbers of lines in oral spines it is necessary to suggest that a fixed, absolute growth increment is not the trigger, but rather that the magnitude of change that will trigger a growth episode in a spine is a function of species, size, and position. A growth increment in a test plate that would trigger a growth episode in an oral spine of a small *Strongylocentrotus purpuratus* would be insufficient to trigger a growth episode in an ambital spine of a large *S. purpuratus*. The absolute change in size of an oral plate needed to trigger a growth episode in an oral spine would be much smaller than the absolute size change needed to trigger a growth episode in an ambital spine.

(4) To tag spines with tetracycline, the tetracycline was mixed with hydrogenated vegetable oil, injected into a hole that was drilled into the spine, and the hole was plugged with a clean *Echinometra mathaei* spine. Tetracycline probably was slowly released for at least 1 wk (Ebert 1985) so it is reasonable to expect that growing surfaces would be tagged. A reasonable conclusion is that the poor success of tetracycline tagging of the spines of *Heterocentrotus mammillatus* was because most of the spines on any particular animal were not growing for at least several days to a week after tagging.

(5) Abrading spines with sandpaper was a simulation of damage caused by sand carried in the surf during storm conditions. Abraded spine surfaces were repaired but new growth lines extending to the milled ring were not formed. It is reasonable to conclude that abrasion is insufficient to cause a complete line to form in spines of *Heterocentrotus mammillatus* that extends from the milled ring to the spine tip.

Discussion. The most important hypothesis of the theory is that spines grow during short episodes and then are not capable of increasing in size until another growth episode. If it can be shown that spines grow in a continuous fashion then the entire theory must be discarded. However, the poor success of tetracycline as

a tag is a strong indication that spines do not show continuous growth.

There is an interesting problem concerning the nature of communication between a test plate and spine in the initiation of a growth episode. Sea urchins of the order Cidaroidae have spines that are known to cease growth: the epidermis dies, growth stops, and the spines frequently become coated with fouling organisms. Before the spine can grow, the entire tip must be shed and when it regenerates it is at a size appropriate for its position on the test and for the size and species of cidarid. The spine is shed following the development of a phagocytic syncytium (Prouho 1887), which is now called Prouho's membrane (Märkel & Röser 1983). The initiation of Prouho's membrane can be due to trauma, such as breaking a spine (Prouho 1887), but it also can be initiated as part of the normal growth process. That is, cidaroids shed spines spontaneously (Cutress 1965). The development of Prouho's membrane is a clear demonstration that there is communication between the test and spines and, furthermore, that communication is local. Not all spines are shed at once on a cidaroid and not all spines of *Heterocentrotus mammillatus* show spontaneous development of new lines (Dotan & Fishelson 1986).

The exact nature of the communication may be associated with the nerve fibers that run below the epidermis of the spine to the spine base and test. There is a subepidermal nerve ring at the base of each spine, which is part of the epithelial plexus (Hyman 1955). Wilbur (1984) has speculated about the stop-and-go nature of what causes layering in mollusc shells and suggests that evidence points to neurosecretory substances. Is this also the link for initiating the development of Prouho's membrane in cidaroids and a growth episode in spines of other urchins?

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