Shell Sculpture and *Ceratostoma foliatum*
by Nicole B. Webster

Thanks in part to the Raymond Archer Marriott Memorial Fund that the PNWSC generously provided me [2014], I completed my PhD 'Development and Evolution of Shell Sculpture in Gastropods' at the University of Alberta with Rich Palmer. This work set the stage to look at the developmental mechanisms that allow snails (and other shelled organisms) to grow shell sculpture. The work was done at the Bamfield Marine Sciences Centre in BC, Canada, focusing on three local muricid species: *Nucella lamellosa*, *Nucella ostrina*, and *Ceratostoma foliatum*.

Some natural history observations

Juvenile growth

Ceratostoma foliatum is the northernmost snail that bears the striking muricid shell pattern. The shell has three broad wing-like (alate) varices on every whorl, which are lined up between whorls and spaced ~120° apart (**Figure 1**). Juveniles start out growing many sharp axial ribs on each whorl, each crosshatched with sharp spiral sculpture (**Figure 1**). As the snails grow, the spacing between axial ribs increases gradually, so that by about the 6-7th whorl, the ribs are arranged into regularly spaced varices with intervarical rounded knobs. The spiral ribs gradually become thicker and more widely spaced, producing the spiral cords. Like many species with periodic varices, *C. foliatum* grows in spurts: the intervarical region and varix are all growth together at once, followed by a longer rest period where they reinforce the new varix and presumably build up resources for the next growth spurt.

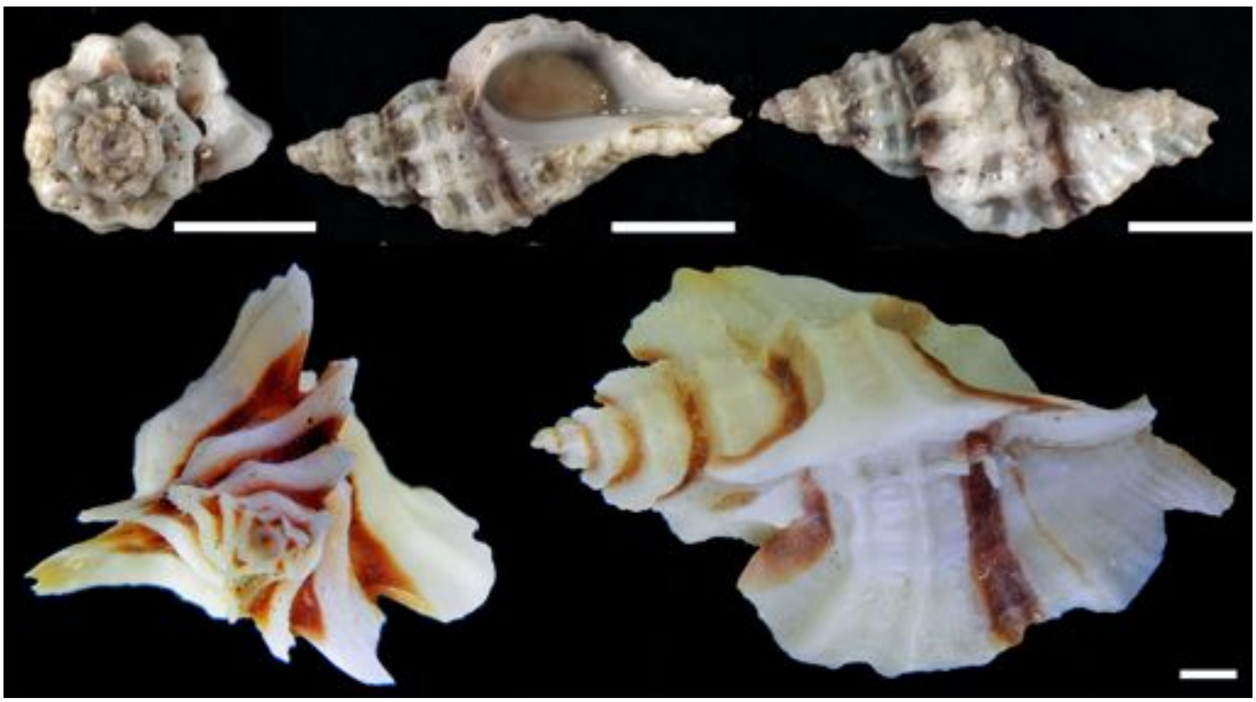

Figure 1. *Ceratostoma foliatum*. Top row: A juvenile snail with ~6 whorls showing how they gradually grow into the adult sculpture pattern. Bottom row: Adult snail showing three varices on each whorl and how each varix grows slightly behind the one above it. All scale bars are 5 mm.

Red tide interrupts growth

During our growth experiments in Bamfield in 2014, there was a red tide (a bloom of the dinoflagellate *Noctiluca* sp.) which completely disrupted shell growth. The snails were being kept in perforated Ziploc containers suspended off the docks, with barnacle (*Balanus glandula*) covered rocks for food. The barnacles sickened and started growing a pink fungus. The snails stopped growing completely, even between varices (unheard of in the wild), and grew a thin lip and labral tooth instead

(**Figure 2C**). We don't know if the effect was as severe on wild *Ceratostoma* at the time, but it does demonstrate some of the effects of a red tide on these predators of filter feeders.

Wild varix variation

Due to the difficulty of finding younger (and thus faster growing) *C. foliatum*, snails were collected both intertidally and by SCUBA from a variety of locations around Barkley Sound. This demonstrated some of the wild variation in varix spacing. Although mathematically, with three varices per whorl you would expect 120° between each varix, the angle is generally a bit smaller than that as each varix fits behind the one in front of it. The mean (of at least three individuals) across populations from seven locations was 111°, with a range from 81° to 140°. Surprisingly, there was a significant difference in varix spacing between some populations, even adjacent ones. Someone would have to look much closer to try to determine if that is genetic drift or natural selection at work.

The funded research

We really don't know how snails produce the complex sculpture patterns, like those we see in *Ceratostoma*. Hans Meinhardt's book, *The Algorithmic Beauty of Shells* (2009), does a great job demonstrating some of the computer models that can simulate natural shell patterns, but whether this relates to how snails do it is very unclear. Beyond a variety of similar molecular hypotheses (Boettiger et al., 2009; Meinhardt and Klingler, 1987) which are difficult to test, another idea to explain how snails can produce regularly spaced sculpture is via physical feedback from the shell (Savazzi and Sasaki, 2004). The idea is that the mantle can feel previous shell sculpture as it lines the aperture and secretes shell. Each varix needs to be partially eroded or it will obstruct the aperture and future growth (**Figure 2A**). This can produce a signal to trigger the production of future shell sculpture. We tested this hypothesis by shaving off the varices on *Ceratostoma foliatum* and letting the snail grow to see if removing the varix had any effect of where new varices grew. We also did the opposite, adding varix 'cues' by gluing an extra varix in front of the aperture to see if that would cause the snails to grow varices earlier than usual.

The results were not as straightforward as we had hoped. When the varix cue was removed, new varices were grown pretty much in position, although significantly further on than the spacing of the previous varix grown on the same shell (**Figure 2B**). This shows that previous varices are not necessary to trigger new varix growth

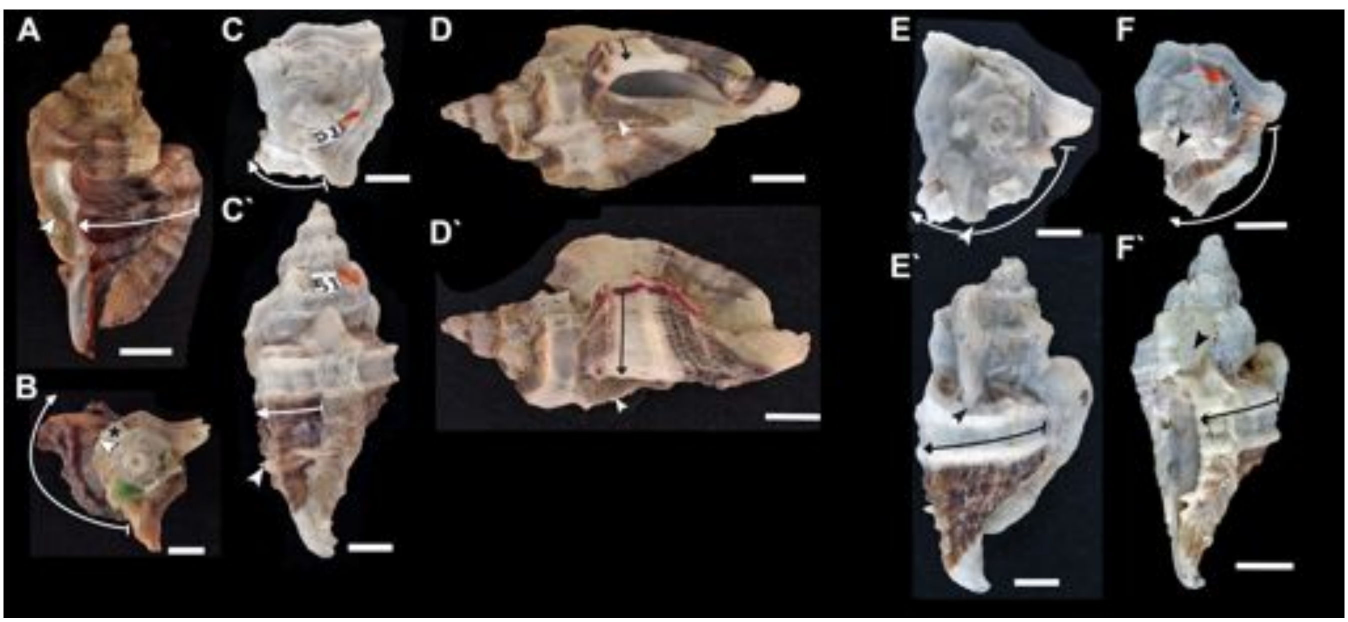

Figure 2. Experimental *C. foliatum*. Curved arrows show amount of growth during the experiment **A**. Arrowhead shows the previous varix in the process of being eroded. **B**. A snail with the previous varix removed (asterisk marks scar where the varix used to be) which still grew a varix in about the right place. The arrowhead denotes the expected location for the varix. **C**. A snail whose growth was disrupted by a red tide. The small ridge is in the wrong location, the arrowhead shows the new labral tooth, which normally only grows with a varix. **D**. A snail whose next varix was coated in Krazy glue (arrowhead). The next varix grew crooked and in the wrong location as a result. **E**. A snail with an added varix (arrowhead) that grew its varix in the expected location despite the artificial cue. **F**. A snail with an added varix (arrowhead) that grew its varix adjacent to the artificial cue rather than in the expected location. All scale bars are 5 mm.

When we added varices to the shell, the shell growth was difficult to interpret. The cyanoacrylate (Krazy glue) used to attach the varices clearly interfered with shell dissolution-a necessary step for the natural varix formation process in the snail. We tested this by lining the next varix with glue in some snails. The snails were never able to remove that varix, and grew angled varices with almost no apertural gap (**Figure 2D**). It was clear that these snails were disrupted by the glue, and the fact that they grew varices adjacent to the glue is likely a result of that. The next year (2014), we glue varices to the shell more carefully, only using glue above the body whorl. In that case, 6/9 snails grew the next varix in the usual location (**Figure 2E**), while 3/9 grew a varix adjacent to the varix that had been glued on **(Figure 2F**).

When putting together the results of both adding and removing a potential varix cue in *C. foliatum*, it is clear that varix cues are neither necessary nor sufficient to trigger new varix production. Some other mechanism, likely a molecular mechanism like those suggested by Meinhardt, is responsible for 'keeping track' of shell growth and triggering varix growth at the right time and place. The fact that varices grew slightly too far apart when the varix cue was missing, or that some grew adjacent to the added varix, does suggest that it's possible that these previous varix cues are used to fine-tune the placement of the new varix just behind the previous one.

It could be very difficult to determine what type of molecular mechanism is responsible for shell patterning, but given the recent rapid advances in molecular tools and methods it may be reasonably possible. I predict that whatever mechanism is responsible will be fairly widespread in molluscs. Bivalves and ammonites in particular also show regularly spaced patterns that would need some sort of internal mapping to grow properly.

References

Boettiger, A., Ermentrout, B., Oster, G., 2009. "The neural origins of shell structure and pattern in aquatic mollusks*". Proc. Natl. Acad. Sci. U. S. A.* 106, 6837-6842. https://doi.org/10.1073/pnas.0810311106

Meinhardt, H., 2009. *The Algorithmic Beauty of Sea Shells*. Springer, Berlin; London.

Meinhardt, H., Klingler, M., 1987. "A model for pattern formation on the shells of molluscs". *J. Theor. Biol.* 126, 63-89 https://doi.org/10.1016/S0022-5193(87)80101-7

Savazzi, E., Sasaki, T., 2004. "Synchronized sculpture in gastropod shells"*. Am. Malacol. Bull*. 18, 87-114.