Developmental stability as the primary function of the pigmentation patterns in bivalve shells?

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ABSTRACT. Most bivalve shells (and other seashells) display complex and highly diverse pigmentation patterns. Crypsis, however, can only explain the presence of complex pigmentation in a few specialised species. Many species live under the sediment and/or their shells are covered by an opaque periostracum. The primary function of the pigmentation patterns, if any, remains a puzzle. I propose here that the pigmentation is intimately associated with the regulation of the growth of the shell to achieve developmental stability. More specifically, I suggest that the pigmentation pattern creates heterogeneity along the shell margin (and incidentally on the rest of the shell) in order to allow the (richly innervated) mantle to position and regulate its shell-growing activity.

KEY WORDS: pigmentation pattern, marine molluscs, sea shells, development, asymmetry, shell growth.

INTRODUCTION

Molluscan shells, particularly seashells, often display eye-catching pigmentation patterns whose sheer diversity and beauty have attracted the attention of collectors and scientists alike. Often the patterns have a striking and complex geometry reminiscent of patterns obtained by mathematical models such as fractals, cellular automata, and Turing reaction-diffusion models. Indeed intensive work on mathematical models has shown how pigmentation patterns can be generated and what allows for their considerable variety (reviewed MEINHARDT, 1995). However the primary function of the pigmentation pattern, if any, is not established and remains an evolutionary puzzle. Secondary functions, such as crypsis, have been occasionally described but are only relevant for a very small number of species.

Here I will review the main known data about pigmentation patterns in bivalve shells and other seashells, and propose a new hypothesis for their primary function. I will suggest that the pigmentation pattern is part of the mechanism for developmental stability, i.e. the mechanism that allows the mollusc to grow a shell corresponding to the optimal shape (e.g. in equivalve bivalves the two valves must be symmetrical). The neural system of the mantle would be involved in sensing the current pigmentation

pattern and use it to position its activity, i.e. deposit new pigmentation and new shell material in a finely regulated way. Some possible means of testing this speculative hypothesis will be also suggested.

SOME EARLIER IDEAS

The present paper will concentrate on bivalves, although most of what will be said here is also valid for other molluscs. The reasons to concentrate on bivalves are as follows. Firstly, bivalves, at least in some families like the Veneridae, exhibit pigmentation patterns whose complexity and diversity rival those in other groups of seashells known for their pigmentation (e.g. gastropods: Conidae, Olividae). Secondly, the pigmentation patterns in bivalves exist in two copies, one on each valve. A crucial observation, which will be discussed further below, is that, at least in some species, there is mirror-symmetry between the pigmentation patterns on the two valves. Finally, because of their abundance, diversity and economic interest, bivalves have received much attention in ecology, population genetics, palaeontology, environmental monitoring and commercial management of stocks. Thus there is a rich body of data on the biology of bivalves, especially on growth and shell formation.

Many bivalves spend the majority of their lives buried in the sediment. Often the shell is completely covered by an opaque periostracum and/or sessile organisms. Furthermore, bivalves have no eyes (although Pectinidae

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have well-developed ocelli). Therefore, contrary to other colourful animals (birds, insects), pigmentation patterns in (most) bivalves cannot function as a signal. A very small number of species that live on the seafloor or other substrate (rocks) may be cryptic but the evidence is limited (COX et al., 1969; CAIN, 1988). Another case of adaptive protection against predation would be the massive colour polymorphism in some species (mostly in the genus *Donax*, possibly *Macoma)*, where predation rate would be decreased by the inability of the predators to develop a search image for all possible morphs (apostatic selection; MOMENT, 1962; ALLEN, 1988; CAIN, 1988). In any case the function of pigmentation pattern as a protection from predation is a secondary function, limited to only very few species. In most species the pigmentation pattern is not cryptic, and there are only few species where the intraspecific variability is sufficient for apostatic selection. The role of the pigmentation pattern in thermoregulation is another possibility, supported by the observations, at least in *Mytilus* species (MITTON, 1977), but again this secondary function can only be adaptive in a few species.

Thus many authors have accepted the idea that pigmentation patterns have no (primary) function at all, and that the high polymorphism in pigmentation pattern, within as well as between species, is a sign that natural selection is relaxed (e.g. SEILACHER, 1972; ERMENTROUT et al., 1986; MEINHARDT, 1995). The idea that pigmentation patterns are not functional, however, seems at odds with their complexity and potential cost of production. Hypotheses for primary functions have been suggested, but none can be considered as adequate. It has been suggested that pigments are waste products of metabolism, secreted in the shell as a means of disposal. This popular idea seems to originate from COMFORT (1951), who stated that in most of the primitive molluscs, shell pigments are almost certainly secreted in the shell as a means of disposal, being either derived from the diet or from unmanageable metabolic residues; no other details or references were given. The hypothesis of pigmentation as waste disposal does not explain why the pigmentation is often so complex. Furthermore, molluscs have a well developed excretory system and it is unclear why some of the metabolic product should be deposited in the shell instead. In some species, the concentration of pigment in the shell correlates with the concentration of chlorophyll (from which the pigment is derived) in the food available in the habitat (UNDERWOOD & CREESE, 1976; see also LEIGHTON & BOOLOOTIAN, 1963). However, even if shell pigments are derived from the food or waste product, their primary function is not necessarily waste. On the contrary, their presence on the shell (as opposed to release into the water) implies that they have some other function. This is in line with the general trend within animals, which is to obtain pigments from their diet, whatever the function of their pigmentation pattern. Another suggestion for the function of pigmentation is that it strengthens the shell

(CAIN (1988), actually referring to the coloration *inside* the shells). Some pigments in the shell are indeed intimately associated with conchiolin, the organic matrix of the shell material (COMFORT, 1951). To my knowledge, however, there is no evidence that pigmentation enhances shell strength.

The structure of this paper is as follows: in the next section, I briefly review what is known about shell growth and about the physiology of pigmentation, the two being developmentally (and, I suggest here, functionally) related. Then I review the work on mathematical models of pigmentation patterns. These models are important because they successfully predict the observed patterns and their variation, and, in the case of bivalves, they suggest that mirror-symmetry between the two valves is difficult to achieve. I then explain my hypothesis and discuss how it relates to the known data. Experimental tests for the hypothesis are also suggested.

WHAT IS KNOWN ?

Shell growth in bivalves

The shell consists of calcium carbonates and an organic matrix, both deposited by the outer layer of the mantle, a large sheet of tissue consisting of two lobes, one lining each valve. The two lobes are connected dorsally. A nerve, called the circumpallial nerve, runs along and parallel to the mantle edge, which is rich in sensory organs, including receptors that may be involved in the regulation of the shell formation (SALEUDDIN, 1979:72). The mantle attaches to the shell along the pallial line, which runs parallel and close to the shell margin. Growth of the shell takes place when the bivalve is respiring and feeding: the valves are open and the mantle edge is protruded. The inner surface of the shell is covered by layers of nacre or other microstructures deposited by the entire outer surface of the mantle. This thickening of the shell will not be considered here; in this paper, growth of the shell refers to the growth in size, at the margin. The above description of shell growth is schematic and there are some variations but they need not be considered here.

The growth of the shell is cyclic, as evidenced by external markings (growth lines with seasonal or annual periodicity, i.e. related to large environmental changes) and internal microgrowth increments (visible under the microscope, with a periodicity of the order of a day). The latter cycle may be caused by periodic valve closure, e.g. at low tides for species living in the intertidal zone (LUTZ & RHOADS, 1980) but there is also evidence that it is uncoupled to environmental variation, suggesting some endogenous mechanism (BERARD et al., 1992).

Pigmentation

The pigmentation is deposited on the shell surface by the mantle edge, during shell growth. Thus, the patterning

of the shells is a graphical representation, in time, of secretory activity along a line of cells, the mantle edge (COMFORT, 1951). Pigmentation patterns usually involve one or two colours (other than white), at least under ambient lighting. Pigments include melanins, pyrroles and porphyrins (COMFORT, 1951) but they have not all been identified.

Patterns of pigmentation on seashells vary from simple to extremely complex. Some shells appear completely white (the natural colour of calcium carbonate) or completely pigmented; others display stripes (parallel or perpendicular to the axis of growth), V-shapes, triangles, waves, spots, blotches, or some combinations of those. Some pigments may not be visible under ambient lighting but only, for example, under ultraviolet light (COX et al., 1969:71). Thus unpigmented or fully pigmented species may well have a more complex pattern than what we see. In fact, I submit that all species have some sort of complex patterns; in non- or completely pigmented species, more complex patterns should appear if appropriate detection methods were known and used.

Mathematical models of pigmentation patterns

The complex geometry of the pigmentation of many molluscan shells has attracted the attention of many theoreticians. Modelling of seashell patterns is facilitated by the fact that growth of the shell and formation of the pigmentation pattern occur only at the margin of the shell, which can be seen a 1-dimensional structure. The final pattern on the shell can therefore be considered as a space-time diagram of the pigmentation process (There are a few exceptions, which need not concern us here; e.g. in species of the genus *Cypraea*, MEINHARDT, 1995:37-39). Pigmentation patterns can be explained by specific changes in the secreting activity of localised groups of cells along the mantle edge (COMFORT, 1951). If the pigment-secreting activity is stable in time, and discontinuous but stable in space, bands perpendicular to the shell margin are produced. If the activity is stable spatially but periodical, bands parallel to the shell margin are produced. If the activity spreads towards adjacent cells, triangles or inverted V shapes will be produced, depending whether the activity is inhibited or not after some delay. More complex patterns can be derived with slightly more complicated rules. Finding these rules and testing their capacity to generate the appropriate pattern by simulation has been rather successful.

WADDINGTON & COWE (1969) seem to have been the first to produce a model and a computer simulation for a pigmentation pattern, based on TURING's (1952) model for morphogenesis by autocatalysis and inhibition processes. Later, cellular automata models have been used (e.g. HERMAN & LIU, 1973; LINDSAY, 1982; WOLFRAM, 1983; GUNJI, 1990; KUSCH & MARKUS, 1996). The model developed by ERMENTROUT et al. (1986) is of special interest as it is based on some hypothetical neural activity in the mantle. Finally, the reaction-diffusion model of morphogenesis was expanded by MEINHARDT (1984, 1995). This model relies on the varying concentration of activating and inhibiting substances, which have typical production, decay and diffusion rates.

An interesting prediction of these models is that the formation of some pigmentation patterns is likely to be chaotic or undecidable. Turing reaction-diffusion models, which rely on variables taking real values, may be chaotic in the sense that a slight difference in the starting or boundary conditions may lead to a very different pattern. In the case of the cellular automata, which rely on variables taking only discrete values, the theory of computation applies. In some cellular automata (class IV of WOLFRAM, 1984) the slightest change in the starting condition will produce a completely different pattern. This property of discrete systems is termed undecidability, or computational irreducibility (WOLFRAM, 1984), meaning that the long term behaviour of such systems cannot be predicted from the initial conditions (this property is related to Turing's Halting Problem). KUSCH & MARKUS (1996) have found evidence for undecidability in several pigmentation patterns of actual seashells. Whether the processes generating the pigmentation patterns are chaotic or undecidable, the consequences that concern us here are the following. Firstly, the diversity of patterns within the population could be explained by small changes in starting conditions and/or rules or parameters. Secondly, in the case of bivalves, the frequent mirror-symmetry of complex pigmentation pattern between the two valves calls for an explanation. Without any sort of control mechanism, it is expected that the patterns on the two valves would diverge, due to small, unavoidable fluctuations. Third, the mollusc may have no control on the details of final pattern, only on its presence and general structure.

Where is the current pigmentation memorised?

The formation of the pigmentation patterns, as demonstrated by mathematical models, depends on adding to the current pattern according to specific rules. How does the mollusc 'know' what the current pattern is? Shell growth, hence pigmentation formation, may last for years and is frequently interrupted, from a few hours per day (e.g. tides) to several weeks or months (e.g. seasons). However, in many cases, the pigmentation pattern is not interrupted by those breaks: it is consistent across the growth marks. Thus when growth resumes, the pigmentation process must resume exactly where it was.

One possibility is that the memory is at the cellular level (LINDSAY, 1982; MEINHARDT, 1995). The pigment-producing cells on the mantle margins may retain their activity state between periods of growth. However, cells of the margin would have to be able to reposition themselves exactly as before; furthermore retaining their activity state may be difficult for periods sometimes lasting up to months. The reaction-diffusion system, as proposed by

TURING (1952) and applied to seashells (e.g. MEINHARDT, 1995) relies on precise variation in the concentration of activating and inhibiting substances. However, living molluscs encounter rather varying environmental conditions (e.g. temperature, water currents) under which it may be difficult to expect any stability, or regular changes in the concentration of the chemical substances as postulated by the model.

Another possibility is that the mantle does not memorise its state, but 'reads' the pigmentation pattern on the shell when needed, by contact chemoreception. Reading the pigmentation from the shell would have many advantages: the information is stable, readily accessible, and there is no energetic cost for maintenance. This 'reading' would be achieved by the rich neural and sensory network of the mantle (e.g. receptors described by SALEUDDIN 1979). ERMENTROUT et al. (1986:374) also proposed the ability of the mantle to taste the old pattern, to explain the alignment to the previous pattern, i.e. in some gastropods where new pigmentation stripes are initiated, on the next round of the shell, at the very position where a stripe appears on the previous round (see illustration in MEINHARDT, 1995:10). The ability to read the previous pattern may also be necessary to allow the stability of the pigmentation pattern despite the growth of the mantle: new cells must appear when the mantle grows (alongside the shell) and this organ is therefore continuously reorganised (cf. LINDSAY, 1982).

Regulation of the growth of the shell

Bivalves close their shells to escape from predators or from temporarily deteriorated environmental conditions. There is a selection pressure for perfect closure and, indeed, most bivalves appear completely sealed when the valves are closed. To achieve this, the two valves must be symmetrical in shape (at least along their margin) in order to close or interlock properly. We can expect some mechanism for developmental stability, otherwise random fluctuations of growth rate at the cellular level could be amplified and cause large (fluctuating) asymmetry in the size and shape of the two valves (cf. EMLEN et al., 1993), preventing proper closure. Environmental perturbations to the shell (erosion, fracture, predation attempts) may also induce an asymmetry unless some co-ordinated repair mechanism could restore the symmetry. Controlling and/or restoring the shape symmetry between the two valves requires a feedback (communication) mechanism between the two mantle lobes. I suggest again that the neural network of the mantle is the major component of this feedback activity. The nervous system is known to interact with the regulation of growth and regeneration of peripheral organs in molluscs (MOFFETT, 1991); it would also be involved in regulating the pigmentation activity (ERMENTROUT et al., 1986) and sensing the previous pigmentation pattern (see above). The existence of a feedback mechanism between the two valves would also

explain the mirror-symmetry of their pigmentation patterns (MEINHARDT, 1995:10); without this feedback the two patterns would diverge rapidly during growth due to the chaotic dynamics of the pigmentation process. The presence of feedback between symmetrical structures to regulate their growth is also discussed by SWADDLE & WITTER (1997) in the case of primary feathers in birds and by EMLEN et al. (1993) on a theoretical basis. Interestingly, the latter authors suggest that feedback between sides should result in growth waves. I propose that this mechanism could explain the endogenecity of the microgrowth observed in seashells (BERARD et al., 1992; see above section on shell growth).

THE HYPOTHESIS

Pigmentation pattern is part of the developmental stability mechanism

My hypothesis is that the pigmentation pattern is intimately related to the growth process, more specifically to the mechanisms that control the growth of the shell in order to achieve the optimal shape (e.g. valve symmetry, in bivalves). I submit that pigmentation and growth are functionally related. Both co-occur temporally and spatially, as the pigmentation is deposited when and where the shell grows (see above). The same (richly innervated) organ, the mantle, would be responsible for growing the shell, depositing the pigment, and sensing the actual shell shape and the current pigmentation.

How could pigmentation pattern and developmental stability be related? The first possibility, as suggested by ERMENTROUT et al. (1986), is that the pigmentation pattern is a recording of the neural activity in the mantle, as an epiphenomenon. It is unclear why this neural activity should be recorded at all, but it is an interesting idea, consistent with my hypothesis that pigmentation and growth regulation are connected. The couplings and feedbacks needed to regulate the growth of the shell may well constitute a system with a non-linear dynamic, the kind of dynamic from which we expect complex and diverse patterns, such as those found in the pigmentation of seashells.

I suggest that the pigmentation pattern is not an epiphenomenon but is used by the mantle as marks to locate position on the shell margin. The need for position marks comes from the fact that the shell margin would otherwise be a uniform one-dimensional structure, along which, however, growth rate must be unequal and adjusted in order to achieve the desired shape. In order to regulate the growth of the shell, the mantle needs to modulate the deposition of new shell material at precise positions along the shell margin. Pigmentation of the shell would provide stable marks that could be read by the mantle at anytime, even after interruption of growth by the endogenous daily rhythm and/or environmentally driven cycles (tides, seasons). In other words, pigmentation would create heterogeneity on the shell that the mantle could use to position

its activity. This primary function would easily be compatible with secondary functions such as crypsis or thermoregulation; it is also compatible with a high degree of intra- and interspecific variability in the pigmentation patterns, and is compatible with the fact that pigments are taken from and vary with the diet.

The role of pigmentation patterns in development would explain their ubiquity. Most species have complex pigmentation patterns, and if not, I suggest that other substances are used for marking the shell surface, which may not be visible to the naked eye. Those substances need not be pigments, but need to be able to mark the shell material in a durable way and to be easily sensed ('tasted') by the mantle. Those pigments or other substances may be waste products and/or taken from the diet. The large variety of pigmentation pattern, both within and between species would be explained by the fact that there may be little selection on the structure of the pattern, as long as it allows positioning. However, some patterns may be more efficient than others for facilitating the mantle to position itself. There might be selection for the kind of regular or complex patterns often seen, but this remains an area of investigation.

The hypothesis presented here remains speculative and needs to be tested. Fortunately some bivalves species can be bred and/or grown in the laboratory (or in aquaculture set-ups), allowing for experimentation. My hypothesis could be tested by perturbing the shell pigmentation and/or the mantle. If the existing pigmentation pattern is altered, perhaps by transplanting part of the shell or directly altering the pigmentation pattern with chemicals, then we expect to observe change in the growth and ultimately the shape of the shell, leading to abnormal asymmetry in shape. Experiments on the mantle could involve the suppression of the communication between the two mantle lobes (by using inhibitors, by sectioning some neural connection – see MOFFETT 1991). Here we would expect abnormal changes in both the pigmentation pattern (asymmetry in species having mirror-symmetry) and the shell shape. Those bivalve species that have mirror-symmetry in their pigmentation pattern may be used to find the actual mechanism of intervalve communication, using a range of neurotoxins or inhibitors and checking whether the symmetry in pigmentation is still achieved or not. In this way, pigmentation patterns may provide a useful handle to study the developmental mechanism of shell formation. Finally, another prediction of the hypothesis that could be verified is the presence of 'hidden' patterns in those species where no pattern is visible under ambient light. Patterns should exist in every species and it would only be a matter of finding a suitable technique to observe them.

In conclusion, I propose to have a new look at the pigmentation patterns of seashells (and its mathematical modelling) on the basis of two ideas. The first concerns the mechanism: the mantle, through its neural and sensory network, regulates the deposition of new pigmentation by reading the previous pigmentation on the shell. The second concerns the function: the pigmentation pattern is related to the developmental regulation of shell growth. My hypothesis is speculative and there are a number of open questions. However, experimental tests can be designed and should provide a better understanding of the evolutionary puzzle of seashell pigmentation patterns. Although centred on (marine) bivalves, my hypothesis may well be valid for other molluscs, marine, freshwater or terrestrial, although it is clear that secondary functions such as crypsis and warning signals have evolved in some species. In those cases, either the pattern for crypsis or warning could be used for growth regulation as well, or non-pigmented markers could be used.

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