Organization pattern of nacre in Pteriidae (Bivalvia: Mollusca) explained by crystal competition

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Bivalve nacre is a brick-wall-patterned biocomposite of aragonite platelets surrounded by organic matter. SEM–electron back scatter diffraction analysis of nacre of the bivalve family Pteriidae reveals that early aragonite crystals grow with their *c*-axes oriented perpendicular to the growth surface but have their *a*- and *b*-axes disoriented. With the accumulation of successive lamellae, crystals progressively orient themselves with their *b*-axes mutually parallel and towards the growth direction. We propose that progressive orientation is a result of competition between nacre crystals at the growth front of lamellae, which favours selection of crystals whose fastest growth axis (*b*-axis) is oriented parallel to the direction of propagation of the lamella. A theoretical model has been developed, which simulates competition of rhombic plates at the lamellar growth front as well as epitaxial growth of crystals onto those of the preceding lamella. The model predicts that disordered nacre progressively produces bivalve-like oriented nacre. As growth fronts become diffuse (as is the common case in bivalves) it takes longer for nacre to become organized. Formation of microdomains of nacre platelets with different orientations is also reproduced. In conclusion, not only the organic matrix component, but also the mineral phase plays an active role in organizing the final microstructure.

Keywords: biomineralization; microstructure; crystallography; shell; nacre; bivalves

1. INTRODUCTION

Nacre is a typical aragonitic microstructure found in molluscs. It is a composite with a layered structure of aragonite platelets (Carpenter 1848) surrounded by an organic matrix (Schmidt 1924) made up of protein–polysaccharide (Towe & Hamilton 1962), which constitutes ca 1% of the total mass (Weiner & Addadi 1997). This provides the material with a brick-wall pattern (Ehrenbaum 1885; Schmidt 1923).

Nacre is exclusive to the Mollusca. It appears in the three major classes (Gastropoda, Bivalvia, Cephalopoda), as well as in the Monoplacophora, though in each of these it has different arrangements. In Gastropoda, nacre crystals stack in towers that finally coalesce by lateral growth (Wise 1970; Nakahara 1983, 1991). Crystals of a same tower have their crystallographic axes co-oriented, with the *c*-axis along the stacking axis (Manne *et al.* 1994). Crystals in different towers have their *c*-axes aligned but the *a*- and *b*-axes have an azimuthal disorder (turbostratic disposition; Schmidt 1923; Wada 1961; Wise 1970). In contrast, nacre in Bivalvia is arranged in terraces, which all grow simultaneously (Schmidt 1924; Wise 1970; Wada 1972). The three crystallographic axes of crystals are co-oriented, with the *c*-axis perpendicular to the nacre surface and the *b*-axis parallel to the local growth direction of the shell margin (Wada 1960, 1961; Weiner & Traub 1981, 1984; Weiner & Addadi 1997; Checa & Rodríguez-Navarro 2005). From among the three main extant classes, *Nautilus*

(Cephalopoda) nacre is probably the least known. Towers and terraces occur simultaneously in adjacent locations (Wise 1970; Mitchel & Phakey 1995). Similar to bivalves, the three main crystallographic axes of *Nautilus* nacre crystals are parallel (Wise 1970; Weiner & Traub 1981).

Several explanations have been given as to how nacre becomes organized differently in each of these mollusc classes. Taylor *et al.* (1969) explained the orientation of the *b*-axis in parallel to the growth direction as the result of currents and concentration gradients in the extrapallial fluid due to mantle movement. Weiner & Traub (1980) found that the intercrystalline matrix is composed of a thin central layer of β -chitin sandwiched between layers of silk fibroin-like proteins, with acidic macromolecules absorbed onto them. The fibre axis of the chitin and silk proteins are perpendicular to each other and aligned with the *a*- and *b*-axes of the aragonite tablets, respectively. Nevertheless, the degree of disorientation of the organic substrate was found to be higher than that of nacre crystals. They proposed that the mineral phase grows epitaxially onto the protein chains of the organic matrix (heteroepitaxial theory). This theory has recently been contradicted by the findings of Nassif *et al.* (2005) who reported the existence of an approximately 3–5 nm thick continuous layer of amorphous calcium carbonate around aragonite platelets. According to them, this layer provides good evidence against the postulated specific interaction between the mineral material and the organic matrix. The observed [001] orientation can be explained by charge interaction with the organic layer.

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Figure 1. (a) Internal surface of the shell of *Pinctada martensii* showing the contact between the outer calcitic prismatic and inner nacreous layers. There is an organic layer (black surface) in between the two layers. Early nacreous tablets are irregular pseudo-hexagonal and begin to grow in the depressed contacts between prisms (inset). Growth direction is towards the top. (b) Early tablets of nacre growing onto a calcite prism of *Pteria hirundo*. Tablets are composed of several crystals, usually forming polycyclic twins. Inset, detail of a polycyclic twin. Growth direction is to the lower right. (c) Diffuse growth fronts of the stepped lamellae of *Atrina pectinata*. Growth direction is to the right. (d) Nacre of *P. martensii* composed of elliptical tablets elongated in parallel to the *a*-axis. Growth fronts are very diffuse. Inset, detail of tablets. Growth direction is to the top. (e) Surface view of the nacre of *Pteria avicula*. Very frequently lamellae are discontinuous and initiate suddenly from crystals of the previous lamellae. Inset, detail of one such crystals growing in two different planes. Growth direction is to the top. (f) Two micrographs of the nacre surface of *Isognomon radiatus* taken at increasing distances from the initiation of the nacre. In the left view (taken at 350 μm) crystals are disoriented, while in the right view (at 2 mm) crystals display a common orientation, with the *b*-axis in the growth direction (right).

Schäffer *et al.* (1997) recognized the existence of many pores (several tens of nanometres across) in the inter-crystalline matrix of the abalone nacre, which they proved to be transparent to ions (similar bridges were previously reported by Bevelander & Nakahara 1969 and Mutvei 1972). They suggested that some of the pores allow crystals to grow from one layer to the next, without the need for a new nucleation event and called this the mineral bridge hypothesis, which implies that tablets from successive lamellae grow epitaxially upon each other (a similar model of growth was formally proposed by Wada 1972). This hypothesis explains how a given orientation is maintained, but not how it arises.

Checa & Rodríguez-Navarro (2005) studied the nacre of pteriod bivalves and concluded that crystal morphology and orientation change from the contact of the nacre with the external prismatic layer towards the interior. Crystals are frequently twinned and disoriented in the *a*-*b* plane at the border. They individualize and orient in the *b*-parallel-to-growth usual mode in a matter of several hundreds of micrometres from the growth edge of nacre. To explain this progressive orientation they proposed that nacre crystals compete at the growth fronts of lamellae, so that those crystals aligned with their longest growth dimension (*b*-axis) in parallel to the growth direction of lamellae survived differentially. Checa & Rodríguez-Navarro (2005) determined orientations of nacre on pole figures derived from X-ray texture diffractometry. In this technique, samples to be measured are squares about 5 $\times$ 5 mm, which provides little resolution since nacre becomes oriented in a much shorter

distance. Therefore, only qualitative (SEM) data supporting the invoked progressive orientation were provided.

Since the issue of nacre organization is still open, we present here a detailed study of bivalve nacre organization by means of microdiffraction techniques. In order to contrast technical data, we have developed a theoretical model for nacre competition in the *a*-*b* plane. All these have been applied to shells of the bivalve order Pterioidea, which are formed by the superposition of a calcitic outer layer of simple prisms and an inner nacreous layer (e.g. Taylor *et al.* 1969; figure 1a). Pteriod nacre is excellently exposed and has been the subject of many classical studies on bivalve nacre, particularly in species valuable in the pearl industry.

Determination of how bivalve and mollusc nacre organizes itself is essential in several respects. Besides the intrinsic interest nacre has in the pearl and medical industries (Camprase *et al.* 1990; Atlan *et al.* 1997), knowing exactly how bivalve nacre organizes itself may greatly influence strategies aiming at designing materials inspired by mineralized biological tissues (Heuer *et al.* 1992).

2. MATERIAL AND METHODS

(a) Material

Specimens appertaining to the species *Pteria avicula* (Calitoban Island, Philippines), *Pteria hirundo* (Mediterranean coast of Andalucía, Spain), *Pinctada margaritifera* (Margarita Island, Venezuela) and *Pinctada martensii* (Uwajima City, Ehime Prefecture, Japan) were studied. All of these appertain to the family Pteriidae (superfamily Pterioidea, Order Pterioidea). Additional data were obtained on members of

Figure 2. (a) Selected area of the nacre close to the limit with the prismatic shell layer of *Pteria hirundo*. Growth direction is to the right. (b) 001 and 010 pole figures for nacre area shown in (a). 001 pole figure indicates that the *c*-axis is perpendicular to the surface and the 010 pole figure implies that the *b*-axis is mainly aligned with the growth direction, although there is a small amount of twinned crystals. (c) Map of the orientation of the *b*-axis with the growth direction for nacre of the area shown in (a). The relationship of colours to angles is indicated in (d). (d) Frequency distribution of angles in the mapped area. (e) Plots of the mean angle formed between the *b*-axis and the growth direction and of the number of lamellae in the 10 sectors distinguished in (c). Left–right distribution of sectors corresponds to that in (c). (f) Selected area of the nacre close to the limit with the prismatic shell layer of *P. martensii*. Growth direction is to the right. Erosion of nacre in the central area has exposed the initial lamellae. (g) Map of the orientation of the *b*-axis with the growth direction for nacre of the area shown in (f). (h) Values and frequency distribution of angles in the mapped area. (i) Plots of the alignment of the *b*-axis and number of lamellae for the eight sectors distinguished in (g).

the families Malleidae, Isognomonidae and Pinnidae, also belonging to the Order Pterioidea. The material is stored in the Departamento de Estratigrafía y Paleontología (Universidad de Granada).

(b) *Electron microscopy*

SEM observation of nacre crystals was carried out both in fractured specimens and in the shells' interiors. Samples were usually observed intact, although in some we removed either the organic (with 5% NaOH for 1–2 h or proteinase K (1 mg ml⁻¹) from 1 to 20 min) or mineral matter (2% EDTA for 10 min) at room temperature. Samples were coated either with gold (Polaron E5000 sputtering) for SEM observation (Zeiss DSM950) or carbon (Hitachi UHS evaporator) for VPSEM (Leo 1430-VP) or FESEM observation (Leo Gemini 1530).

(c) *Electron back scattering diffraction analysis*

This technique is based on the fact that when an electron beam interacts with a crystalline material under low angle incidence, the backscattered electrons are diffracted by the material forming what is known as an electron back scatter diffraction (EBSD) pattern. This pattern depends on the orientation of the crystal lattice of the backscattered electron interaction volume and implicitly contains information about the space group of the crystal structure (Randle 2000). Indexing the pattern gives information about both the orientation of the crystal lattice and the crystalline phase. Using the scanning capabilities of an SEM (Philips XL-30), data from different positions can be either integrated into an orientation map or processed to obtain pole figures, pole plots or rocking curves resembling those obtained by X-ray diffraction. Details about the EBSD technique are described by, e.g. Schwarzer 1997.

Two specimens each appertaining to the species *P. martensii* and *P. hirundo* (Pteriidae) were studied. The organic matter was removed from the samples prior to analysis. Although the samples were studied without any conductive coating, no excessive charging of the specimen was observed and the quality of the patterns was acceptable in almost all cases.

3. RESULTS

(a) *SEM observations*

Early seeds of nacre form on the organic sheet intermediate between prisms and nacre and are usually found on the relatively depressed interprismatic areas (figure 1*a,b*). With time, nacre crystals progressively invade the prism centre (figure 1*a*). The growth front of this initial lamella can be defined as diffuse, since it is a band of variable width within which crystals in very different stages of growth can be found, the more advanced ones being more incipiently grown. Initial nacreous tablets are usually large in size (up to 20 µm; figure 1*a,b*) and have extremely varied shapes, from dendritic (figure 1*a*) to euhedral (figure 1*b*). Twinning is also common (figure 1*b*). Variable orientation of tablets is the common pattern (figure 1*b*).

After the first nacreous lamella completely covers the prismatic layer, the typical stepped mode of growth of bivalve nacre initiates. The step size between lamellae may vary between shells and within the same shell, as well as the degree of diffusion of their growth fronts (figure 1*c,d*). A common case in Pteriidae is that a lamella initiates suddenly from another underlying lamella by lateral

elevation of particular crystals (figure 1*e*). In this way, the number of lamellae counted across different transects from the initiation of the nacreous layer to a given lamella can vary.

With accumulation of lamellae (i.e. at a distance of 100–150 µm from the prism–nacre interface) tablets become idiomorphic, monocrystalline and smaller-sized (typically 2–8 µm; figure 1*c,d,f*). Their morphology may be extremely varied. Most commonly nacre crystals are bounded by {110} rhombic faces, with their crystallographic *b*-axis along the largest diagonal; their acute corners are sometimes truncated by {010} faces (figure 3*b*). Depending on the existence and relative development of {100} and/or {010} faces crystals may acquire pseudo-hexagonal (figure 1*c*), pseudo-octagonal (not observed here) or, very rarely, rectangular (figure 1*f*) outlines. A good compilation of crystal shapes and their relationship to crystallographic faces is provided by Wada (1972) (see also Wise 1970 and Checa & Rodríguez-Navarro 2005). Crystals are either equidimensional (figure 1*c*) or elongate along the *b*-axis (figure 1*f*), although Wada (1972) reported that *P. martensii* is able to seasonally (July–October) deposit pseudo-hexagonal crystals which are elongated along the *a*-axis. Finally, nacre crystals can be rounded and we report here the rare instance of oval crystals elongated along the *a*-axis in *P. martensii* (figure 1*d*).

The degree of orientation of tablets also increases towards the nacre interior. Tablets become evenly oriented with the *b*-axis parallel to the local growth direction of the margin at a variable distance of 500–800 µm from the prism–nacre boundary (figure 1*f*). This orientation is maintained throughout the rest of the shell interior.

(b) *EBSD analysis*

Maps of crystal orientations were made on shells of *P. hirundo* (figure 2*a*) and *P. martensii* (figure 2*f*). In each we selected one area of 500 × 300 µm, including the contact with the overlying prismatic shell. A step size of 1.8 µm was selected, which yielded 46 296 measurements per map. The number of measurements with a confidence index greater than 0.1 was greater than 70% in both cases. Pole figures for 001 and 010 reflections indicate a good alignment of *c*-axes perpendicular to the nacre surface and *b*-axes perpendicular to the nacre–prism boundary, with a small incidence of polycyclic twinning (about 11%; figure 2*b*). We have also mapped the spread angle of the *b*-axis with the growth direction (*b*-axis alignment), which is shown in variable colours (figure 2*c,d,g,h*). In both cases, the angle decreases progressively from the prism–nacre boundary towards the shell interior, although there are small areas in which the angle remains high. This does not apply to the centre of the mapped area of *P. martensii* because here nacre is eroded and the early 3–4 lamellae remain exposed. We have divided the upper band of each map in sectors (shown in figure 2*c,g*) and calculated the mean alignment of the *b*-axis with the growth direction for each sector. The mean angle in the sector closest to the prismatic layer is around 40° in both cases. This angle later diminishes progressively in the dorsal direction, to stabilize around 6° at 275 µm in *P. hirundo* (figure 2*e*). The plot of the mean number of lamellae per sector indicates that the number of lamellae increases in the same direction. The figure is similar in *P. martensii* in which the

Figure 3. (a) Model of competition between hypothetical nacreous tablets in the plane of the lamella (i.e. that containing the crystallographic *a*- and *b*- axes). The boundaries between growing crystals are drawn along the intersections between equivalent growth increments. Only those crystals whose longest diagonals (*b*-axes) are parallel to the growth direction of the lamella (to the left) survive preferentially. (b) Geometrical reconstruction of boundaries between crystals using the same procedure as in (a). We have reproduced several cases in the growth front of a lamella of *P. hirundo* (lower right photograph). Nacre tablets are rhombic ($\{110\}$ faces, with slightly developed $\{010\}$ faces), with the *b*-axis along the longest diagonal. A sketch is also provided for clarity. In all selected cases (framed) the contacts between crystals fit quite closely with those produced by the model.

mean angle diminishes to 9° at the fourth sector (150–200 μm; figure 2i). This value later remains constant, although the two more internal values are not representative due to defective preservation of nacre in this area. The number of lamellae also increases with decreasing angle, although at a lower rate than in *P. hirundo*.

4. DISCUSSION

(a) Organization and progressive alignment of nacre crystals

Our pole figures obtained from EBSD agree with Laue measurements of Wada (1961) and Wise (1970) and pole figures of XRD of Checa & Rodríguez-Navarro (2005). In brief, bivalve nacre is well organized in the sense that crystals demonstrate a common orientation of their three crystallographic axes, with the *c*-axis perpendicular to the growth surface and the *b*-axis parallel to the growth direction of lamellae.

SEM observations led Checa & Rodríguez-Navarro (2005) to infer that orientation of nacre crystals is progressive from the prism–nacre boundary towards the shell interior. Maps and mean values of the *b*-axis alignment obtained from EBSD analysis (figure 1c,e,g,i) indicate that the orientation of the *b*-axis is almost random (turbostratic disposition) near the boundary with the prismatic layer, with mean angles around 40° (complete disorganization would be 45°). This angle later diminishes inwards towards the dorsum to stabilize at around 6° in *P. hirundo* and 9° in *P. martensii*. These values probably represent complete organization of nacre. In conclusion, it is confirmed that alignment of the *b*-axis of nacre crystals is progressive and increases with the

piling up of lamellae. Due to defective preservation, the first 3–4 layers of nacre remain exposed in the central area of the map of *P. martensii*. As a consequence alignment remains very poor.

In the map of *P. hirundo* (figure 2c) and in the upper band of that of *P. martensii* (figure 2g) records of angle values different from the general trend come in clusters (i.e. mainly red areas on the purple background), which can be interpreted as microdomains in which nacre tablets are evenly oriented. Microdomains were well documented in the nacre of the mussel *Mytilus edulis* by Hou & Feng (2003).

(b) Competition between tablets

The mode of growth of nacre crystals has been well established (Wise 1970; Wada 1972; Mutvei 1977; Nakahara 1991). In brief, at the growth front of an adorally extending lamella incipient nacre crystals soon acquire their maximum height along their crystallographic *c*-axis, which is perpendicular to the lamella and later extend sideways within the plane of the lamella (i.e. the *a*–*b* plane) until impinging on one another. At this moment, crystals must compete with each other within this plane during growth. Geometrical models of competition between crystals (Grigor'ev 1965) state that the boundaries between competing crystals run along the intersections between synchronous growth increments (figure 3a). It is assumed that the band width for a given growth increment is always the same for all crystals. In aragonite, growth along the *b*-axis is about 1.6 times faster than along the *a*-axis (aragonite cell unit dimensions $a = 4.959 \text{ \AA}$, $b = 7.968 \text{ \AA}$, e.g. Klein & Hurlbut 1998). In a growth front composed of ideally rhombic crystals

Figure 4. Theoretical simulation of competition between nacre crystals at the growth fronts of lamellae and epitaxial growth of crystals onto those of the preceding lamella. Three cases with different values of inclination are shown. Crystals are coloured from pale blue to dark pink according to the angle formed by their b -axis (long diagonal) with the growth direction of the lamella (bottom to top direction). (a) For $I=0.2$, a markedly diffuse front is obtained. A general trend is obtained only after 400 layers have superimposed (see histograms). Except for layer 0, crystals which are not oriented with their b -axis parallel to the shell growth direction organize in clusters (microdomains). These tend to vanish with increasing alignment. (b) $I=0.4$ produces an intermediate growth front. The number of layers needed to obtain the same basic features as in the preceding case is about one order of magnitude lower. Microdomains also form. (c) A clearly neat front is obtained at $I=0.58$. Alignment is very fast and is produced with about two orders of magnitude less layers than for $I=0.2$. Microdomains are apparent in layers 2 and 4 and almost absent in layer 8. The graphs below each case show data of alignment versus layer number for five different runs (the curves corresponding to the cases shown above come in the same colours as the histograms). The range in the final alignment and number of layers needed to obtain a good alignment decreases with increasing I . For $I=0.2$, between *ca* 250 and 850 layers are needed for the alignment value to be less than 10° and there is a case (red curve) in which alignment is close to 20° in layer 1000. When $I=0.58$ a similar alignment value is produced after only 4–6 layers and alignment fluctuates very slightly (6 – 7°) in layer 10. The case of $I=0.4$ is intermediate.

approaching this relationship (i.e. with their b -axis along the largest diagonal), competition unavoidably leads to survival (selection) of those crystals whose longest diagonal is aligned in the direction of propagation of the lamella (figure 3a). This simple competition model reproduces the boundaries between nacre crystals in actual shells quite well (figure 3b). Relative crystal size causes displacement of the contact towards the smallest crystal, the more the higher the difference in size, but does not influence the shape of the contact.

In brief, the normal figure in bivalve nacre is elongation along the b -axis, which explains why it organizes with the b -axis parallel to the local growth direction of the shell. The rare or exceptional record of equidimensional crystals or of those having a major a -axis and the fact that they are

only secreted seasonally (Wada 1972), explains why orientations other than the above mentioned have never been recorded in bivalves.

(c) Theoretical simulation

In order to explore the implications of the model we have devised a computer program, which reproduces the effects of competitive growth, lamellae superposition and epitaxial growth in actual shells. Rhombic crystals grow on an x - y surface of 21×21 square cells, each cell sizing 25 pixels. Growth direction is upwards (increasing y). Only one crystal seed may appear at a random position within a cell. Once a crystal appears, it grows from a seed by 1 (crystallographic a -axis) $\times$ 1.62 (crystallographic b -axis) during each growth interval, the direction of crystal axes

being randomly determined. In this way, the space is finally filled with crystals. In order to reproduce the situation of diffuse *versus* linear growth fronts, a parameter of inclination (I) has been introduced in the following way. We assume that there is a linear trend between the time of appearance of a given crystal (S) and its position along the Y -axis of the diagram (Y): $S = -I \times Y$. A small S value implies that the difference in time between crystals located at different positions along a transect perpendicular to the growth front is small or, in other words, new crystals appear when former crystals are still incipiently grown. In this way, a wide growth front composed of growing crystals is formed. Therefore, decreasing I implies more diffuse growth fronts.

Piling up of layers is also simulated. The direction of a crystal on a new layer is determined by that of the crystal of the previous layer onto which it begins to grow. In this way, we reproduce the effect of epitaxial growth, which is well established in bivalve and gastropod nacre (Manne *et al.* 1994; Schäffer *et al.* 1997). Finally, the program also provides the histogram of angle frequency at intervals of 10° and the mean angle between the b -axis and the growth direction (alignment of b -axis).

Simulations carried out at a large range of I values always produce a good alignment of theoretical crystals with their largest diagonals parallel to the growth direction of lamellae (figure 4), although there are differences as to the required number of layers and the final arrangement. Small I values produce diffuse growth fronts, which is by far the most common case in bivalve nacre. In this situation, selection is weak and a good alignment is produced only after a large number (hundreds) of layers have accumulated. The case of $I=0.2$ has been illustrated in figure 4a. In the diagram of alignment of the b -axis versus number of layers we have plotted data from five different runs. It can be appreciated how the alignment finally stabilizes at an extremely variable number of layers (400 and 900 layers in the cases shown) and, interestingly it may take values well above 0° . Another remarkable feature is that crystals with orientations different from the main trend arrange in clusters (microdomains). These tend to disappear with the accumulation of layers, although some still persist in layer 800.

Progressively greater I values produce less diffuse growth fronts, although this situation is seldom found in actual nacre. The cases for $I=0.4$ and 0.58 are illustrated in figure 4b,c, respectively. Increasing I causes faster alignment of crystals due to more intense selection. Plots of b -axis alignment show a continuous descending trend and do not clearly stabilize. Values below 10° are obtained between 50 and greater than 100 layers ($I=0.4$) and 4–6 layers ($I=0.58$; see bottom panels figure 4b,c). Microdomains are still apparent in both cases, but tend to disappear earlier and to be smaller in size with increasing I .

It is important to stress that our theoretical layers only extend the screen height, thus being incomparably shorter than actual ones, which extend ideally from the dorsum to the contact with the prismatic layer. Since selection intensity is also dependent on the extent of the lamella (i.e. the time the growth front is active) results similar to the theoretical ones would be obtained with a much lower number of lamellae in actual shells.

In conclusion, our theoretical model predicts good alignment of b -axes in parallel to the growth direction for

any value of I . Increasing I values imply a reduction in the number of layers needed to produce such alignment and a final alignment angle closer to 0° , as well as a reduction in the persistence and size of microdomains.

5. CONCLUSIONS

SEM–EBSD data imply that bivalve nacre organizes progressively from the crystallographic viewpoint from the boundary with the prismatic layer inwards towards the shell with accumulation of successive lamellae. We have concluded that alignment is obtained by competition between crystals at the growth front of superimposing lamellae. The theoretical model devised predicts that competition and epitaxial growth of crystals onto those of the underlying lamella cause good alignment of the b -axis with the growth direction even when the growth fronts of lamellae are diffuse. Selection and, hence, alignment becomes better and faster when growth fronts are neater. Interestingly, the model predicts the formation of microdomains in nacre, which is progressively inhibited as selection becomes more intense due to the development of very neat fronts.

Checa & Rodríguez-Navarro (2005) explained that crystal selection within the a – b plane is not possible in gastropod nacre because growth proceeds at the edge by vertical stacking of polycrystalline nacreous tablets (Wise 1970; Nakahara 1983, 1991). Crystals of the same lamella in adjacent towers grow separately and only impinge on each other when the growth front has advanced well beyond their position. Therefore, they cannot influence the orientation of crystals growing at the margin. This explains why gastropod nacre has a random orientation of a - and b -axes (Wise 1970; Nakahara 1983 and own data).

Towered growth in gastropods is probably a strategy addressed to rapidly thicken the shell at a short distance from the aperture. This is probably not necessary in bivalves as both valves fit closely. Therefore, the two patterns of growth of nacre lamellae can be seen as major constructional constraints associated with the different body plans. If this is accepted, the three-axial crystallographic organization of bivalve nacre would merely be a by-product of its stepped mode of growth (independently of whether it confers mechanical advantages).

In summary, our model of organization by crystal competition explains the progressive nature of nacre orientation with accumulation of lamellae, the consistent orientation of the b -axis in parallel to the growth direction of lamellae, the formation of microdomains in bivalve nacre and the differences in orientation between bivalve and gastropod nacre. A better knowledge of cephalopod (*Nautilus*) and monoplacophoran nacre may also allow future testing of the predictions of the model. Another possibility is to disrupt the original pattern over a given area by, e.g. implanting nacreous bivalves with neutral or non-nacreous crystalline substrates.

The heteroepitaxial model of growth of the crystalline phase onto the organic matrix (Weiner & Traub 1980, 1984; Weiner *et al.* 1983; see §1) does not fit well with the progressive organization of the bivalve nacre (unless it is invoked that a certain time span is also required for the organic matrix also to organize) and is apparently in conflict with our model of organization by competition. A possibility to reconcile both views is to admit that both

heteroepitaxy and selection by competition superimpose in bivalve nacre, the latter having a prevalent effect. As commented on in §1, the recent finds of Nassif *et al.* (2005) exclude the possibility of heteroepitaxy. Accordingly, our model of crystal selection by competition would remain as the sole explanation of organization in the *a*-*b* plane.

Due to its biomechanical and biomedical interest, bivalve nacre is by far the most intensively studied mollusc shell microstructure. A prerequisite in biomimetic studies is the exact knowledge of the process of formation, which requires characterization of the growth front where the control on crystal morphology and orientation is exerted. In this sense, the application of novel techniques, such as SEM-EBSD, has allowed us to characterize the pattern of crystallographic ordering with an unprecedented detail. The basic tenet in biomineralization is that the organic matrix is responsible for the high degree of organization of mollusc shell microstructures. For example, it is known that the organic matrix is essential in determining the aragonite/calcite nature of the mineral phase (Belcher *et al.* 1996; Falini *et al.* 1996) and crystal shape and orientation in nacre (Fritz *et al.* 1994; Belcher *et al.* 1996). But our study reveals that the mineral phase also plays an active role in organizing the biomaterial.

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