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MOLLUSCAN SHELL PIGMENTS: AN *IN SITU* RESONANCE RAMAN STUDY

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ABSTRACT

Using Resonance Raman microspectrometry we identify polyenes as shell pigments in 13 gastropod, 1 cephalopod and 4 bivalve taxa. The pigments are either isolated polyenes or polyenes bound to other molecules. Polyenes are present in coloured parts of the shell of all investigated taxa. Pigments differ between taxa and there is no trivial relationship between colour, pigment and taxon. The same colour in different taxa may be due to different pigments; different colours in the same taxon are due to different pigments; different colour saturation is due to different concentration of the same pigment. The pigment polyenes are unsubstituted chains of 8–13 conjugated double-bonds (16–26 carbon atoms) in the chain, with terminals incorporating $-CH_3$ groups. This is the first demonstration of polyenes as shell pigment in the Mollusca. We also identify a carotenoid as the external, yellow pigment in *Cypraea moneta*.

INTRODUCTION

Mollusc shells have vivid colours and intricate patterns, but the pigments are practically unknown. Comfort's (1951) observation that 'the coloured substances which occur in molluscs offer a remarkably wide and largely unworked field to the biochemist' is regrettably still true. For the sake of clarity, we use the term 'colour' to designate a visual impression, and the term 'pigment' to designate compounds, known or unknown, having colour.

Comfort's (1949, 1950, 1951) identification of porphyrins in mollusc shells figures prominently in later discussions. Most studies (Takagi & Tanaka, 1954; Takagi, 1955; Sawada, 1958, 1961; Koizumi & Nonaka, 1970a, b; Iwahashi & Akamatsu, 1994) emphasize pearls or shells of pearl-producing molluscs, and only few (e.g. Akamatsu *et al*., 1977; Dele-Dubois & Merlin, 1981) explicitly link identified pigments to pearl or shell colour. Considerable work on opisthobranchs (e.g. Cimino & Sodano, 1993; Cimino, Fontana & Gavagnin, 1999; Cimino & Ghiselin, 1999) reveal accumulation, modification and synthesis of very diverse, usually polyunsaturated molecules in soft tissues. Many are accumulated in the mantle tissue and serve defence (Cimino & Ghiselin, 1999). Some of the compounds are coloured, but it is unknown whether similar compounds and processes form shell pigments in other molluscan groups. Models of shell-pattern formation (Meinhardt, 1995) emphasize reaction-diffusion systems, but do not discuss which compounds react and diffuse.

In this study, we identify polyenes (molecules with multiple conjugated carbon–carbon single- and double bonds) as pigments in mollusc shells by Resonance Raman MicroSpectrometry (RRMS). Raman spectrometry is a non-destructive technique providing dynamic and structural information on molecules. It has been applied to analyses of protein dynamics, drug interactions, single cells, bacteria and viruses, and it provides a characteristic signal for certain molecular bonds. The position and intensity of peaks in a Raman spectrum relate to the composition and structure of molecules. The positions of the maxima depend on specific vibrational modes (for example carbon–carbon single-bond or double-bond stretching), and are very sensitive indicators of differences in the molecular structure. Confocal Raman microspectrometry analyses sample volumes of a few cubic micrometres. In this paper, we generate Resonance Raman spectra of shells, demonstrate that the spectra are due to pigments, and identify the pigments as polyenes. This is the first identification of the same class of pigments causing coloration of shells across crown groups of the Mollusca clade.

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MATERIAL AND METHODS

We investigated individuals of the exemplar taxa in Table 1, chosen for strongly coloured shells, by *in-situ* RRMS on complete shells to provide molecular vibrational information. Under certain experimental conditions, the Raman scattered signal is enhanced due to the resonance effect that occurs when the excitation energy is close to an absorption band of the observed molecules. We selected the 514.5 nm wavelength radiation from an argon/krypton ion laser to provide a good signal/noise ratio, and the laser power at the sample was reduced to 4 mW to minimize possible sample deterioration. We used a microscope equipped with a 50x objective, focusing the incident laser beam on the sample and retrocollecting the scattered light that was analysed by a spectrometer with a single monochromator (600 gratings per mm), coupled to a CCD detector. We used a confocal Jobin-Yvon T64000 Raman spectrometer with a pinhole placed behind the focal plane of the microscope. This enhances resolution by reducing light coming from outside of the focal plane and gives a depth resolution along the optical axis of a few μm. The confocal system is commonly used in the study of small samples with a volume of few cubic micrometres (Tabaksblat, Meier & Kip, 1992). In our case, it also improved the ratio between the Raman signal intensity and the fluorescence signal intensity that can occur in shell samples. The integration time varied between 3 and 240 s depending on the sample. The Raman spectra in the figures have been corrected for background fluorescence and the base-line is shifted for clarity.

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Table 1. Investigated taxa, shell colour, and wavenumbers of peaks.

Sample	Taxon	Colour	R_1 (cm^{-1})	R_4 (cm^{-1})	N_1	N_4
1	*Conus marmoreus* Linnæus, 1758	White	–	–	–	–
2	*C. marmoreus* Linnæus, 1758	White	–	–	–	–
3	*C. marmoreus* Linnæus, 1758	White	–	–	–	–
4	*C. marmoreus* Linnæus, 1758	Black	1122	1507	11.9	12.0
5	*C. marmoreus* Linnæus, 1758	Black	1122	1507	11.9	12.0
6	*C. marmoreus* Linnæus, 1758	Black	1122	1507	11.9	12.0
7	*Architectonica perspectiva* (Linnæus, 1758)	Brown, dark	1123	1510	11.6	11.5
8	*A. perspectiva* (Linnæus, 1758)	Brown, pale	1123	1510	11.6	11.5
9	*A. perspectiva* (Linnæus, 1758)	White	–	–	–	–
10	*Nerita albicilla* Linnæus, 1758	Black	1133	1522	9.0	9.9
11	*Cymbula oculus* (Born, 1778)	Black	1118	1500	13.2	13.4
12	*Strombus luhuanus* Linnæus, 1758	Black	1120	1502	12.5	13.0
13	*Cypraea moneta* Linnæus, 1758	Blue	1122	1504	11.9	12.6
14	*Mytilus edulis* Linnæus, 1758	Blue	1098	1484	29.8	18.0
15	*Chlamys senatoria* (Gmelin, 1791)	Brownish	1130	1516	9.9	10.6
16	*C. mauritana* Linnæus, 1758	Brown, dark	1120	1501	12.5	13.2
17	*C. mauritana* Linnæus, 1758	Brown, pale	1125	1513	11.1	11.1
18	*Helix aspersa* Müller, 1774	Brown	1132	1522	9.5	9.9
19	*Nautilus pompilius* Linnæus, 1758	Brown	1112	1492	15.9	15.4
20	*N. albicilla* Linnæus, 1758	Brown	1133	1522	9.3	9.9
21	*Oliva miniacea* (Röding, 1798)	Brown	1133	1524	9.3	9.7
22	*C. oculus* (Born, 1778)	Brown	1118	1500	13.2	13.4
23	*Smaragdia viridis* (Linnæus, 1758)	Green	1135	1528	9.0	9.2
24	*C. senatoria* (Gmelin, 1791)	Orange	1135	1526	9.0	9.4
25	*Mitra mitra* (Linnæus, 1758)	Orange	1134	1525	9.2	9.5
26	*O. miniacea* (Röding, 1798)	Orange	1133	1524	9.3	9.7
27	*Spondylus princeps* Broderip, 1833	Orange	1133	1523	9.3	9.8
28	*C. senatoria* (Gmelin, 1791)	Purple	1128	1511	10.3	11.4
29	*Hiatula diphos* (Linnæus, 1771)	Purple	1126	1510	10.8	11.5
30	*C. senatoria* (Gmelin, 1791)	Red	1130	1517	9.9	10.5
31	*Cypraea aurantium* Gmelin, 1791	Red	1130	1518	9.9	10.4
32	*Entemnotrochus adansonianus* Crosse & Fischer, 1861	Red	1133	1520	9.3	10.1
33	*S. luhuanus* Linnæus, 1758	Red	1131	1518	9.7	10.4
34	*C. senatoria* (Gmelin, 1791)	Yellow	1125	1509	11.1	11.7
35	*C. moneta* Linnæus, 1758	Yellow	1158	1522	6.3	10.9
C	β-Carotene (Fluka 22040)	Orange	1154	1514	119	9.9

R_1 designates wavenumbers of carbon–carbon single bond stretching vibration, R_4 wavenumbers of carbon–carbon double bond stretching vibration. N_1 is the number of conjugated double-bonds, expected in a linear polyene, calculated from the observed single-bond Raman shift (R_1); these values are determined from the equation $N = B_1/(R_1 - A_1)$ with $A_1 = 1082$ and $B_1 = 476$ (Schaffer *et al.*, 1991). N_4 is the number of conjugated double-bonds, expected in a linear polyene, calculated from the observed double-bond Raman shift (R_4); these values are determined from the equation $N = B_4/(R_4 - A_4)$ with $A_4 = 1438$ and $B_4 = 830$ (Schaffer *et al.*, 1991).

We failed in repeated attempts to extract and isolate pigments from shells and were unable to use other spectroscopic methods.

RESULTS

Figure 1 shows Resonance Raman spectra of six spots, recorded between 500 cm^{-1} and 4000 cm^{-1}, alternating from coloured (black) and uncoloured bands, along a growth line of *Conus marmoreus*. The spectra of the three black spots are qualitatively identical, the spectra of the three white spots are identical, but the spectra of black spots differ from those of white spots. The peaks at wavenumbers 701, 705 and 1085 cm^{-1} (marked 'A' in Fig. 1) are due to aragonite. We find them in all samples containing aragonite, whether coloured or not, but since the peaks are very narrow, they do not perturb other peaks. The strongest peaks are found in the region 800–1700 cm^{-1}. Peaks around 1100–1130 cm^{-1} and 1500–1530 cm^{-1} marked '–C–C–' and '–C=C–' in Figure 1 (referred to in the following as R_1 and R_4, respectively), are due to carbon–carbon single-bond and double-bond stretching vibrations. The presence of these two enhanced peaks indicates the spectra are due to either carotenoids or polyenes (Schaffer *et al.*, 1991; Britton *et al.*, 1997; Oliveira *et al.*, 1997; Tarantilis *et al.*, 1998) and, for comparison, we show the resonance Raman spectrum of β-carotene. The medium-intensity peaks above 2000 cm^{-1} are due to overtones and combination tones of the strong –C–C– and –C=C– fundamental peaks. We observe additional weak-intensity peaks in the frequency range 1000–1030 cm^{-1} and 1290–1300 cm^{-1} and will address them in the discussion.

Raman spectra of differently coloured individuals of *Chlamys senatoria* are shown in Figure 2 in addition to those of three spots near the margin of the same individual of *Architectonica perspectiva*. Identical features were observed in the 800–1700 cm^{-1} range, i.e. aragonite peaks and carbon–carbon

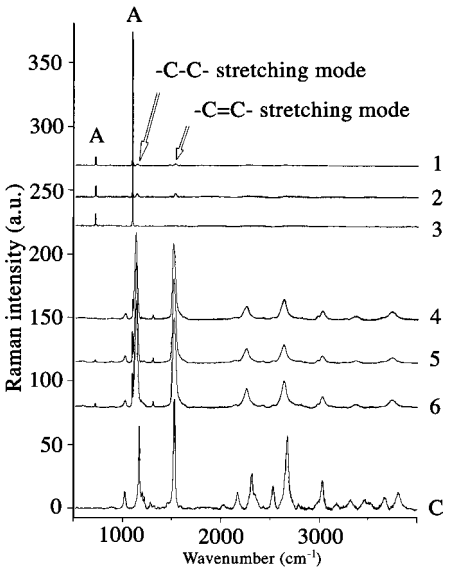

Figure 1. Raman spectra in arbitrary units of samples 1–6 (see Table 1), and C. 1–6 are six points along approximately 2 cm of one growth line of a *Conus marmoreus*. 1–3 are from three white points, and 4–6 from three black points between the white. C is a β-carotene standard. The baselines are shifted to facilitate comparison. The signal at wavenumbers 701, 705 and 1085 cm^{-1} (marked 'A') is due to aragonite. Peaks marked '–C–C–' and '–C=C–' are due to carbon–carbon single-bond and double-bond stretching vibrations, respectively.

single- and double-bond stretching bands around 1120–1130 and 1500–1530 cm^{-1}, respectively. It is worth noting that while the spectra keep the same overall structure, the positions of the strongest peaks differ slightly between samples with different colours. Figure 3 shows the frequency dependence of R_4 versus R_1 for the pigments analysed and, for comparison, the predicted Raman shift of resonantly coupled modes for polyenes with different chain length and experimental results for a series of carotenoids.

Table 1 reproduces the wavenumber R_1 and R_4 of the main peaks observed in the Raman spectra. We adopt the labelling

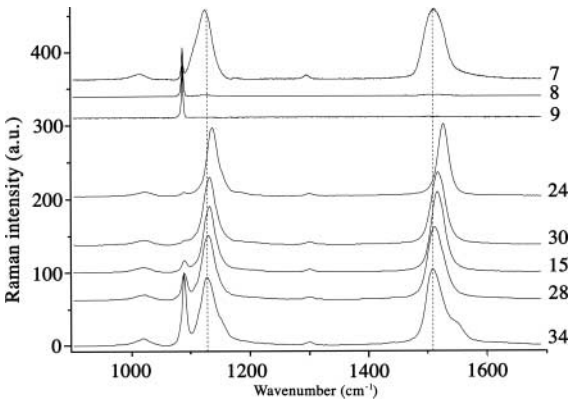

Figure 2. Raman spectra of samples 7–9, 15, 24, 28, 30 and 34 (see Table 1). 7–9 are three spots near the margin of the same individual of *Architectonica perspectiva*, 7 is from the dark brown band at the suture, 8 from the pale brown base colour of the shell, and 9 from a white band between the others. 15, 24, 28, 30 and 34 are differently coloured individuals of *Chlamys senatoria*. The baselines are shifted to facilitate comparison, the first axis gives wavenumber in cm^{-1}, the second axis shows intensity in relative units.

of the peaks used by Schaffer *et al.* (1991) to facilitate the discussion of our results relative to the formulae presented by these authors. In Table 1 we include the calculated number of conjugated double bonds N_1 and N_4 in linear polyenes given, respectively, the wavenumbers R_1 and R_4 of the carbon–carbon single-bond and double-bond peaks, calculated from the formula derived from analyses of synthetic references by Schaffer *et al.* (1991):

$$N_1 = \frac{476}{R_1 - 1082} \qquad \text{and} \qquad N_4 = \frac{830}{R_4 - 1438}$$

DISCUSSION

We invariably get persuasive Raman spectra from coloured parts of shells. Samples 1–6 from *Conus marmoreus* are taken along a contemporaneous growth line in one individual (Fig. 1). They were deposited by the same individual, at the same time, under identical environmental conditions. We record identical spectra from coloured spots, and only aragonite peaks in spectra from uncoloured spots. Apart from the aragonite peaks, the spectra from coloured spots must be due to pigment or compounds with the same localization as the pigment in the shell. Our Raman spectra are characterized by strong peaks attributed to carbon–carbon single- and double-bond stretching vibrational modes that are main features of molecules possessing a central polyene chain (as in β-carotene) with different substitutions. Based on the Raman spectra, we propose that the shells contain pigments with the general structure similar to polyenes and carotenoids. Raman spectra alone cannot resolve specific molecular structure, but we will show in the following that the detailed analysis of salient features can reveal important structural information allowing us to distinguish between these two groups of pigments.

Figure 1 shows the Raman spectrum of β-carotene. The main peaks were assigned by Saito & Tasumi (1983) from results of the normal-coordinate calculations for all-trans-β-carotene. The strong peaks at 1154 and 1514 cm^{-1} are due to stretching of the C–C bonds and C=C bonds. We also observe a fine structure above the peak at 1154 cm^{-1} specific to the β-carotene. The medium-intensity peaks at 1191, 1213 and 1270 cm^{-1} are due to C–C stretching mode with one carbon bonded to a methyl group and the weak-intensity peak at 1445 cm^{-1} to the deformation of the methyl group bonded to one carbon along the chain. We did not observe any of these bands from the investigated pigments in Figures 1 and 2, suggesting that the shell pigments contain polyene chains, as do carotenoids, but do not have $-CH_3$ groups attached to the chain. The Raman spectrum of β-carotene molecules is also characterized by another strong-intensity peak near 1008 cm^{-1}. This peak is attributed to the rocking mode of the methyl groups bonded to a carbon along the chain. The assignment of this band was experimentally confirmed by Okamoto *et al.* (1984) and Kim, Furukawa & Tasumi (1997) who reported, respectively, on the vibrational studies of tetradesmethyl-β-carotene and all-trans-19,19′,20,20′-tetranor-β,β-carotene (both characterized by a central polyene chain without methyl group attached to it) and showed the Raman spectra without strong bands in the 1000–1100 cm^{-1} frequency range. We observe a weak-intensity peak in the frequency range 1290–1300 cm^{-1} that is usually attributed to the CH in-plane bending mode (Schaffer *et al.*, 1991; Kim *et al.*, 1997). The large weak-intensity band around 1018 cm^{-1} ($\pm$20–25 cm^{-1}) in our spectra could be assigned to the rocking mode of a methyl group as terminal function of the chain or part of it. Further chemical investigations are needed to verify this point.

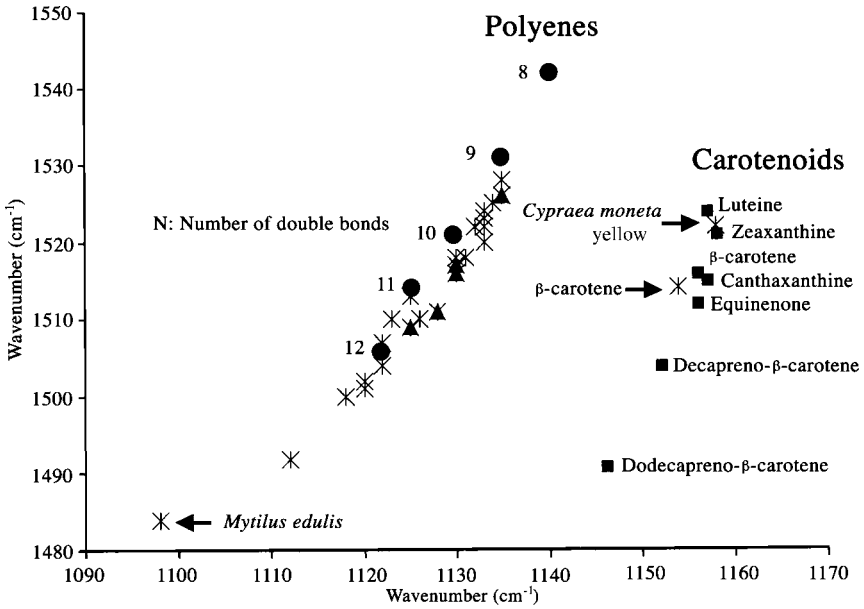

Figure 3. N_1, the expected number of conjugated double-bonds calculated from the wavenumber of the carbon–carbon single-bond peaks, plotted against N_4, the expected number of conjugated double-bonds calculated from the wavenumber of the carbon–carbon double-bond peaks. The line corresponds to $N_1 = N_4$. Filled-in symbols are data from *Chlamys senatoria*. Points for *Mytilus edulis* and β-carotene are outside the figure.

All the peaks from coloured parts of the shells can be satisfactorily explained as due to polyenes and aragonite. Polyenes can be pigments, coloured compounds, as found in parrots' feathers by Veronelli, Zerbi & Stradi (1995), although polyenes are unusual in organisms. As we observe polyene peaks only from coloured parts of the shell (the peaks are absent from uncoloured parts), we conclude that the shell pigment in every sample contains polyenes.

Figure 2 shows samples 7–9, which give stronger signals from more saturated spots on an individual shell of *A. perspectiva*, but no qualitative difference (same shift positions) between spectra of more or less saturated spots are observed. This demonstrates that the Raman signal is due to the same pigment, and the intensity corresponds to colour saturation, and probably to pigment concentration.

Peak positions for polyenes and carotenoids shift slightly depending on the length of the polyene chain, functional groups attached to the chain, cis- or trans- conformation and/or which other molecules (e.g. proteins) are bound to the molecules producing the spectra (Saito & Tasumi, 1983; Schaffer *et al.*, 1991, Kim *et al.*, 1997; Weesie, *et al.*, 1995). In order to better understand the relationship between peak positions and polyene chain length, we calculated the expected number of conjugated double bonds N_1 and N_4 in linear polyenes assuming respectively the wavenumbers R_1 and R_4 of the carbon–carbon single-bond and double-bond peaks. The results are presented in Table 1. We point out that Schaffer *et al.* (1991) worked on unsubstituted all-trans polyenes with $N = 3–12$ double bonds, but their formula is based on the best fit of a line for $N = 8–12$. Consequently, we may find inconsistent results for higher or lower N. The two calculated numbers of double-bonds differ markedly from each other only for two samples, *Mytilus edulis* and the yellow pigment in *Cypraea moneta*, and for β-carotene. For the latter, we calculate 119 conjugated double-bonds from R_1 and only 10 from R_4 (9 is correct), but we know that β-carotene violates the requirement of an unsubstituted polyene by having $-CH_3$ groups attached to the chain.

Figure 3 shows the experimental frequency dependence of R_4 versus R_1 and the predicted Raman shift of resonantly coupled modes for an all-trans polyene with different chain length and experimental results for a series of carotenoids. The experimental Raman shifts appear linear and in good agreement with the predicted frequencies for polyenes. The pigment of *M. edulis*, presented in Figure 3, differs from the others by being out of the linear prediction, but Schaffer *et al.* (1991) demonstrated the validity of the linear extrapolation only up to a 13–ene. By using this plot we can estimate the effective conjugation length of C=C along the polyene and suggest most of the pigments are unsubstituted all-trans chains of 8–13 conjugated double-bonds (thus 16–26 carbon atoms in the chain) with terminals incorporating $-CH_3$ groups, except for *M. edulis* which should contain more than 15 double bonds. We caution against a rigid interpretation of the number of conjugated double-bonds, since this assumption does not take into account that the pigment is actually embedded in the biological matrix which could distort chains. This study is the first demonstration of polyenes as shell pigment in the Mollusca.

The yellow pigment from the outside of *C. moneta* (indicated by arrows in Fig. 3) differs from the above pattern. The resonance Raman spectrum (not shown) of the yellow part of the shell shows a strong peak at 1158 cm^{-1} and fine structure at 1190, 1213, 1268 and 1444 cm^{-1}, that are specific features of carotenoids. In addition, we observed the strong-intensity peak at 1006 cm^{-1} (14 cm^{-1} width) attributed to the rocking mode of the methyl groups bonded to a carbon along the chain. Our data for the yellow pigment in *C. moneta* correspond to those of zeaxanthine, but further investigations are needed for a definitive identification. Among our samples, only this shell has a pigment, containing a polyene chain with $-CH_3$ groups attached to the chain, that is a carotenoid. We note that *C. moneta* has a blue pigment on the inside of the shell, which we interpret as an unsubstituted polyene, and a yellow carotenoid on the outside.

Figure 2 shows Raman spectra of five differently coloured individuals of *Chlamys senatoria*, each with strong uniform

colour throughout the shell. They were all collected by the same diver on the same day off Ko Racha, South of Phuket, Thailand. Individuals with the usual brownish colour live on the open sandy bottom, and brightly coloured individuals live in crevices or under rock (S. Patamakanthin, personal communication). The colour difference may be due to genetics, different food, the formation of specific pigment–protein complexes (as described for carotenoids by Weesie *et al*., 1995) or a charge redistribution mechanism along the chain contributing to the shift of vibrational modes and to the modification of colour with the same pigment. The polyene pigments differ in number of polyconjugated double-bonds (tentatively 9–11). Underwood & Creese (1976) demonstrated a relationship between the amount of available food and amount of porphyrin pigments in shells. If shell pigments are modified from the food, it is possible that the different colours and pigments in *C. senatoria* are due to different habitat. *Chlamys senatoria* is a filter feeder and detritus available under coral or rock may differ in composition. The observed shifts of the C=C stretching and C–C stretching mode for the purple (number 28 in Table 1) and yellow (number 34 in Table 1) shells are less than 4 cm^{-1} for each mode. As the number of double bonds is estimated to be the same value ($N \sim 10$), the different colour may be due to different functional groups on the pigment or to interactions with the organic matrix. We do not have evidence supporting either explanation. The interesting point is that individuals, presumably from a single population of a single taxon, may produce markedly different colours and pigments.

If a given colour is due to a specific pigment, we would expect samples with the same colour in Table 1 to have peaks at wavenumbers within ± 2 cm^{-1} of each other, roughly corresponding to the resolution of our experimental setup, and thus to infer the same number of conjugated double-bonds. This is not the case for any colour, where we have several samples.

Raman microspectrometry is a very sensitive and useful technique. The spectra can be recorded *in situ*, that is on a background of numerous unknown organic compounds that are shielded by the choice of wavelength. With a proper setup, the method can also be applied to live or at least freshly dead samples. Furthermore, the probe can be focused, so it is possible to record spectra from a few cubic micrometres volume within translucent samples. We can estimate the number of polyene molecules contributing to the spectra as:

$$\frac{(\text{Vol. analysed}) \cdot (\text{Sp. gravity}) \cdot (\text{Conc. w/w}) \cdot (\text{Molecules/mole})}{\text{Mole mass}}$$

Let us assume that we analyse approximately 10 cubic micrometres of shell, that mollusc shells have a specific gravity of 2.8 g/cm^3, and an average polyene with 10 polyconjugated double-bonds (20 carbon atoms in the chain) has a mole mass of 700 gmole. Avogadro's number, $N_A = 6.023 \cdot 10^{23}$ molecules/mole. Underwood & Creese (1976) found approximately 2–11 ppm porphyrin in shells of *Austrocochlea constricta* (Lamarck). Comfort (1951) implied that carotenoids are at least more sparse than porphyrins and he would likely have isolated polyenes with carotenoids (substituted polyenes). If we assume shells contain less than 2–11 ppm polyenes, we record spectra from less than 50,000–285,000 molecules. This will be a helpful estimate to develop techniques for extracting pigments.

Mollusc shells also have pigments other than polyenes. Comfort (1951) discussed several without elaborating on taxa. Carotenoids are common in mollusc soft tissues (Fox, 1976; Vershinin, 1996), but Comfort (1951) repeated that they are absent from shells; we note that Comfort (1951) and many others dissolved shells in acids that decompose carotenoids, precluding their identification. Koizumi & Nonaka (1970a) isolated yellow pigment from pearls, and tentatively identified one component as flavoxanthin, a carotenoid. Carotenoids have been identified by Raman spectrometry as shell pigments in *Strombus gigas* by Dele-Dubois & Merlin (1981) and by the Gemological Institute of America (S. Elen, personal communication). Akamatsu *et al*. (1977) identified carbohydrates as part of the pigment in yellow pearls, and Sawada (1958, 1961) recorded porphyrin as part of the yellow pigment of pearls from *Pinctada fucata martensii* (Dunker, 1850). Creese & Underwood (1976), Underwood & Creese (1976), and Jones & Silver (1979) identified porphyrins in shells from two gastropod taxa.

Comfort (1949, 1950) identified porphyrins in many mollusc shells, but did not mention which do not have porphyrins. Comfort's work has often inspired a search for porphyrins in shells, rather than identification of pigments. Comfort (1951) noted '... the pattern of distribution [of porphyrins] in an individual species may or may not coincide with the visible pigment,' and not all shells without porphyrins are white. For example, *Cypraea* and *Marginella* shells, of which only a few contain porphyrins (Comfort, 1951), are usually strongly coloured. This suggests that porphyrins occur in mollusc shells, but not necessarily as pigments. We have not demonstrated the absence of porphyrins and do not refute the above results, nor have we demonstrated the absence of other pigments. We have demonstrated that polyenes are present in coloured parts of the investigated shells, absent from the uncoloured parts, and more concentrated in parts with higher colour saturation. The colour of a given shell is possibly due to several compounds, at least one of which is often a polyene with an unsubstituted chain of 8–13 conjugated double-bonds, provided chains are in all-trans configuration.

Knowing the pigments and their properties may allow us to understand their biological function. We do not subscribe to Comfort's (1951) view that '... shell pigments are almost certainly secreted in the shell as a means of disposal...' Molluscs have excellent excretory organs, and incorporating waste into structural supports seem implausible, particularly when secretion is episodic and confined to a thin layer at the exterior of the shell. In his review of carotenoids in molluscan soft tissues, Vershinin (1996) discussed carotenoids as constituents of cell membranes and how they affect membrane rigidity. Vershinin (1996) questions the association of carotenoids and proteins in soft tissues, whereas Comfort (1951) stresses the association of mollusc shell pigments and proteins. Mollusc shells contain proteins and carbohydrates that are in part responsible for shell structure morphology and crystallography (Hatchett, 1799; Falini *et al*., 1996), and polyene pigments may have a structural function in the formation of the matrix in outer shell layers.

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