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COLOUR CHANGES IN THE SHELL OF THE TREE-CLIMBING BIVALVE *ENIGMONIA AENIGMATICA* (HOLTEN, 1802) (ANOMIIDAE)

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ABSTRACT. - Substrate dependent colour changes in the shell of the tree-climbing anomiid bivalve *Enigmonia aenigmatica* are described and discussed.

INTRODUCTION

Enigmonia aenigmatica (Holten, 1802) is a small anomiid bivalve found in the mangrove forests of southeast Asia. As the name implies, it has intrigued zoologists for nearly two centuries by its unique life habits of climbing about on the trunks, branches and leaves of mangrove trees, more in the manner of a gastropod than a bivalve (Yonge, 1957), and also by its many varieties of shape and two distinct colour morphs, reddish purple and golden yellow, some of which have in the past been described as separate species (Colour Plate 19 II, Figs A-E). Gray (1859) described four varieties. While the morphological varieties have been treated as such by most workers in this century, the colour morphs persisted as separate species as late as 1975 (Berry, 1975). Morton (1976) found no differences in the anatomy of the colour morphs and treated them as one species. The different distribution of the two colour morphs has long been noticed: the golden variety (*E. aenigmatica*), which has a smoother shell, is found on the leaves of mangrove trees whereas the purple variety, which also has a rougher surface conforming to the surface texture of the bark or other substrates it grows on, is found on the trunks and branches of mangrove trees and on other intertidal hard substrates such as wooden, concrete and steel structures, and has traditionally been named *E. rosea*. However, no attempts have previously been made to explain the reasons for the existence of two colour morphs on different substrates.

On numerous trips to the mangroves the senior author had noticed that many of the golden shells on the leaves had been purple or red at younger stages persisting on the shell, indicating that a change in colour had taken place after the animal had settled on the leaf. This article describes the results of experiments conducted to test the hypothesis that the distribution of the two colour morphs can be explained by the fact that red or purple colour does not develop in the shells of *Enigmonia aenigmatica* growing on the leaves of mangrove trees,and conversely, that golden coloured shells which migrate from leaf to any other substrate will change to purple.

MATERIALS AND METHODS

Experiments To Demonstrate Change In Colour

E. aenigmatica were collected from mangroves at Kranji and Sungei Buloh on the northwest shore of Singapore. Small to medium sized animals were selected for use in the experiments because of their faster growth rates. Larger shells were used to obtain extracts for analysis of shell pigments. Every care was taken not to injure the animals intended for use in experiments. Those with purple shells were removed from trunks or branches by cutting off a piece of bark to which the animal was attached. In the case of animals growing on leaves, the whole leaf was picked. The animals on the pieces of bark or leaves were put into seawater in large crystallising dishes in the lab. They would then detach themselves from the substrate and crawl up onto the sides of the dish from where they could easily be removed without injury. To transfer the animals to the experimental substrates, the following procedure was used: The animals were placed left valve down in shallow seawater in a Petri dish. The foot would soon emerge through the byssal sinus in the right valve. The new substrate, stone, wood or leaf, was then brought into contact with the foot and the animals would attach themselves. For growing *Enigmonia* on leaves, small *Avicennia alba* saplings that were collected in the mangroves and grown in sand in plastic containers, were used. During the experiments, the animals were subjected to an arbitrary "tidal" regime of 8 hours submerged and 16 hours dry. This was achieved simply by lifting the stones or pieces of wood or containers, in which the *Avicennia saplings* were growing, out of the water. The salinity of the water was adjusted to 26 ppt. A suspension of a tropical strain of *Isochrysis galbana* was added as food. Three experimental regimes were used:

1. Golden coloured shells removed from leaves of *Avicennia alba* were grown on large granite chips or bricks.

2. Golden shells removed from leaves of *A.alba* were grown on dead wood collected from mangroves.

3. Purple shells removed from trunks or proproots of mangrove trees, or from stones or concrete walls, were grown on leaves of *Avicennia alba.*

The experiments were started on 7 June 1988. For each experimental setup 20 animals were used. The experiments ended on 20 September. Average growth increase (max. length) for all three experimental setups combined for the three months 20 June to 20 September was 6.9mm (SD=1.9). The fastest growth recorded was an increase of 13mm (from 8mm to 21mm) and the slowest was 3.5 mm during the same period. Overall mortality during the period was 13%.

Sections were made of shells used in the experiments. They were embedded in a mixture of Epon 812, MNA and DMP30 and thin sections were cut using a Buehler Isomet low speed saw with a diamond wafering blade.

Pigments were analysed for in extracts made separately of golden and purple shells by dissolving them in 10% HCl acid. The strongly purple-pigmented conchiolin matrix was discarded and the solution was filtered to remove debris. The solution of the purple shells had a strong reddish purple colour whereas that of the golden shell was colourless. Extracts were subjected to ultraviolet radiation to test for fluorescence and a UV-visible light spectrum was also obtained in a Beckman DU-64 spectrophotometer.

The extracts were also quantitatively analysed for 13 elements in an atomic absorption spectrometer in order to ascertain whether the difference in colour could be due to differences in metallic ion contents. The solubility of the pigments in ether, sodium carbonate and and ammonium hydroxide, besides HCl, was also tested.

RESULTS

Results Of Tests For Pigments

UV irradiation. Extract of the red/purple variety of shells showed strong red fluorescence under ultra violet irradiation. Extract of the golden variety showed no fluorescence. Red fluorescence is characteristic of porphyrins (Comfort, 1949).

Absorption spectra obtained for extracts of the two colour morphs are shown in Fig.1. Extract of purple shells showed strong absorption from about 390 nm to about 420 nm with a maximum peak at 407 nm and subsidiary peaks at longer wavelengths matching quite well those given for uroporphyrins by Krugelis-Macrae (1956). Extract of golden shells gave no absorption peaks at wavelengths ranging from 210 nm to 680 nm.

The purple pigment extracted from purple *Enigmonia* shells was insoluble in ether, excluding the possibility that the porphyrin in question is a protoporphyrin, mesoporphyrin, deuteroporphyrin or coproporphyrin, all of which are ether soluble. (Lemberg & Legge 1949). It was, however, soluble in ethyl acetate, sodium carbonate, ammonium hydroxide as well as 10% HCl providing further evidence that the pigments in the shell of the purple morph of *E. aenigmatica* are indeed uroporphyrins (Lemberg & Legge, 1949; Krugelis-Macrae, 1956).

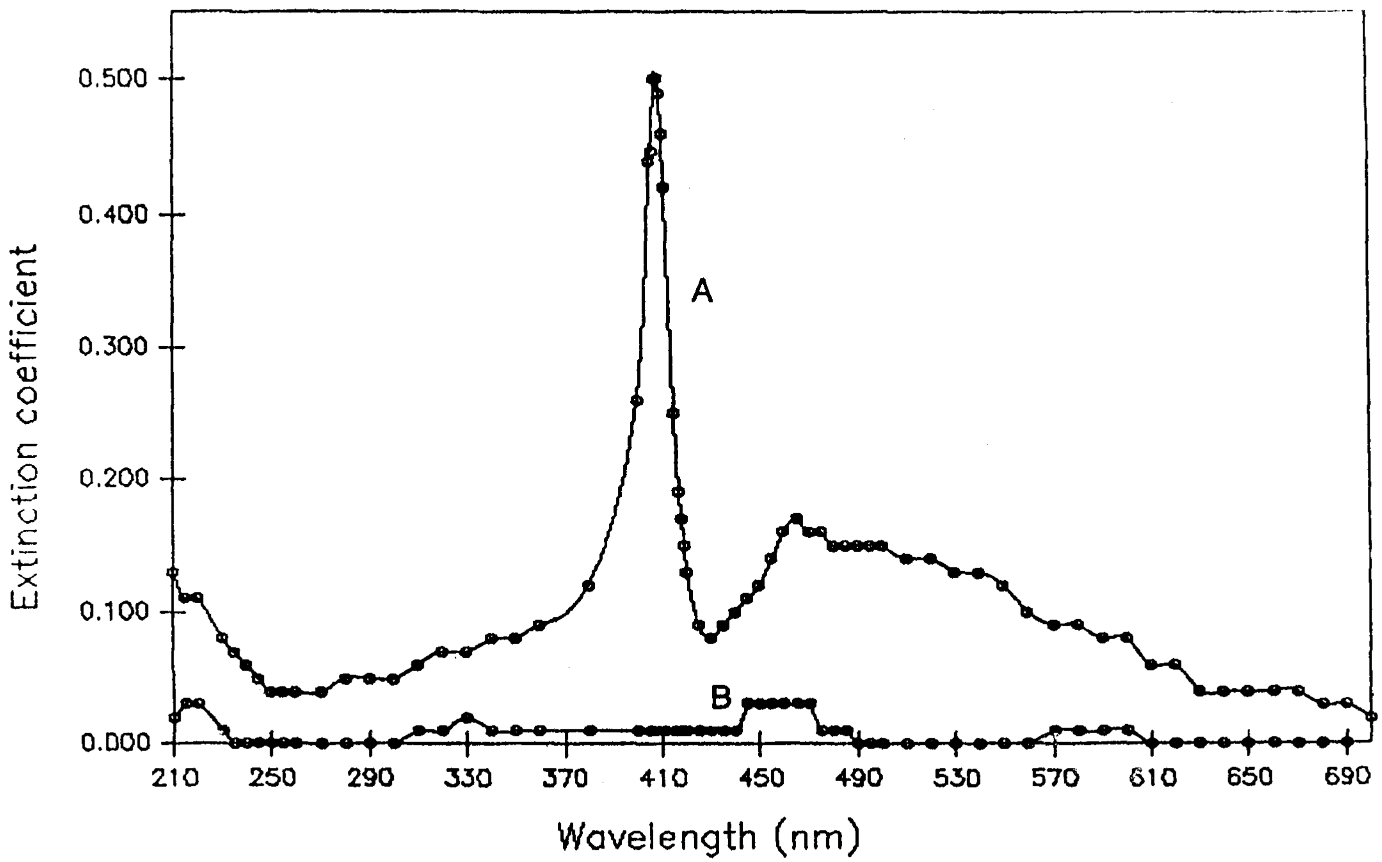
Atomic absorption analysis for 13 elements (Ca, Sr, Na, Mg, Si, P, Zn, Cu, Fe, Mn, V, Co, Cr) showed all the elements to be present in almost equal amounts in both colour morphs, indicating that the difference in colour is not due to differences in metal ion content.

Results Of Experiments

Results for experiments 1 and 2.-- All the golden shells from leaves of *A. alba* grown on stones, bricks or wood turned red/purple. The first indication of change in colour from golden was a red tinge of some of the shells that started showing after about one month. At the end of the experiment all the surviving shells (87.5%) had turned purple (see Colour Plate 19 II G). Control shells of the purple variety remained purple. The results were the same for shells grown in ambient light and those grown in the dark. In the field it is sometimes possible to distinguish shells that have changed colour from golden to red because the part that grew on the leaf is smoother than the part that grows on the trunk and it may also have a slightly lighter colour as shown by the specimen in Colour Pl. 19 II G. A section of the edge of a shell that changed from golden to purplish red is shown in Colour Pl. 19 II I. This clearly shows that the red shell layers are added to the inside surface of the shell as well as to the margins.

Results for experiment 3.- All the purple shells grown on *Avicennia alba* leaves turned from purple to golden colour from their margins as shown by the shell in Colour Pl. 19 II F. The golden shelled controls remained golden. Unlike the golden shells that turn red all over

Fig. 1. UV-visible light spectra for extracts of shells of *Enigmonia aenigmatica*. A: extract of purple shells. B: extract of golden shells.



when moved to a substrate other than leaves, the red shells remain red and turn to a golden colour from the edge of the original shell only as shown in Colour Pl. 19 II F. Sections of shells that changed from purple to golden show that a colourless or yellowish layer, is added to the general inside surface of the shell, but this is masked by the original red or purple shell. Many golden shells growing on leaves have a small purplish patch at the umbo as mentioned by Morton (1976). Close examination reveals that in many cases this is the original reddish-purple shell that settled initially on the stems of seedlings low down in the intertidal and later crawled up the trunks and branches to reach the leaves whereupon it later grew up as a golden shelled individual.

DISCUSSION

It has long been known that the upper (left) valves of the shell of *Enigmonia aenigmatica* are coloured red to deep purple, as shown in Colour Pl.19 II. A, B, and G, on all substrates other than leaves of mangrove trees, on which they are of a golden colour. Comfort (1948) reported porphyrins from the shell of *Enigmonia* and we have shown here that the colour of the red to purple shells is probably due to uroporphyrin, which is deposited both in the organic conchiolin and the inorganic parts of the upper (left) valve of the shell. The lower valve always remains colourless as shown in Colour Pl. 19 II C and E.

E. aenigmatica is highly motile, and crawls from its initial settlement sites, which are lower down in the intertidal on seedlings of mangrove trees and pieces of wood and other hard substrates (unpublished observations), onto trunks and the leaves of mangrove trees. The juveniles are red to purple in colour, but when they reach the leaves the colour of the shell turns to golden. The initial red/purple shell remains on the umbo of the later stages indicating the size at which the shell reached the leaf. However, not all the animals reach the leaves; those on the trunks remain purple. Further, some crawl from leaf back to trunk and back again several times. Each time an individual moves from trunk or branch to leaf or leaf to trunk, the colour of the shell will change and a record of the number of such movements will be left in the shell as bands of coloured and colourless/golden shell layers, provided they stay on the same substrate for long enough. One such record is shown in Colour Pl. 19 II K, where the individual has apparently moved four times between leaf and other substrates. Studies of marked *E.aenigmatica* (unpublished) have revealed a surprising amount of movement of this kind. We have found marked individuals in the mangroves that have moved to other trees several metres distance.

Our observations and experimental results make it clear that the purple colour does not develop in *E. aenigmatica* growing on leaves of mangrove trees. This statement must be further qualified, however, to include only those animals growing on the underside of the leaves, where by far the majority of *E. aenigmatica* growing on leaves are found. In the few cases where we have found *Enigmonia* growing on the top surface of leaves, the shells are usually tinged with red. We offer two hypotheses to interpret these results.

The first hypothesis is that the change in colour from purple to golden is due to environmental factors only and not controlled by the animals themselves. Uroporphyrins can be destroyed and rendered colourless by oxidation, and we think that the most likely explanation for the absence of the colour in shells of *E. aenigmatica* growing on the underside of leaves is that oxygen, or some other oxidizing agent evolving from the numerous stomata on the underside of the leaves destroys the uroporphyrin before it can be deposited in the shell. Our observations of the red tinge of shells growing on the upper surface of leaves, where there are few if any stomata, appears to support this hypothesis, by indicating that some uroporphyrin had been deposited in them. The same process may be responsible for the bleached look we have observed in some shells of *Littoraria* spp., especially *L. conica*, found on leaves of *Avicennia* spp. and other mangrove trees.

On the other hand it is clear that *E. aenigmatica* has some control over the deposition of pigment in the shell. This is demonstrated by the fact that the lower (right) valve always remains colourless even when the upper valve is dark purple. We cannot therefore exclude the possibility that the changes in colour that we have observed in the field and demonstrated in experiments are due to an ability of *Enigmonia* to switch deposition of uroporphyrin into the shell on and off depending on whether it is growing on leaves or other substrates.

It is tempting to speculate on the adaptive advantages to *Enigmonia aenigmatica* of the colour morphs. Most other anomiids have colourless or whitish shells and the advantage to an animal that sits on a dark tree trunk of having a dark coloured shell rather than a white one, appears fairly obvious. The golden shells on the leaves also seem to be better camouflaged than if they were dark in colour, and both colour morphs therefore may offer some protection against visual predators. On the other hand the pigmented shell of *Enigmonia* may offer protection against the harmful effects of light that could easily penetrate the very thin shell of *Enigmonia* if it were colourless. *Enigmonia* is a filter-feeding bivalve ingesting large amounts of one-celled algae. As these are broken down in the gut, chlorophyll will be freed which, if activated by light, will give off extremely poisonous oxygen radicals (Hendry, 1988). Further experiments are underway to try to answer these questions.

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