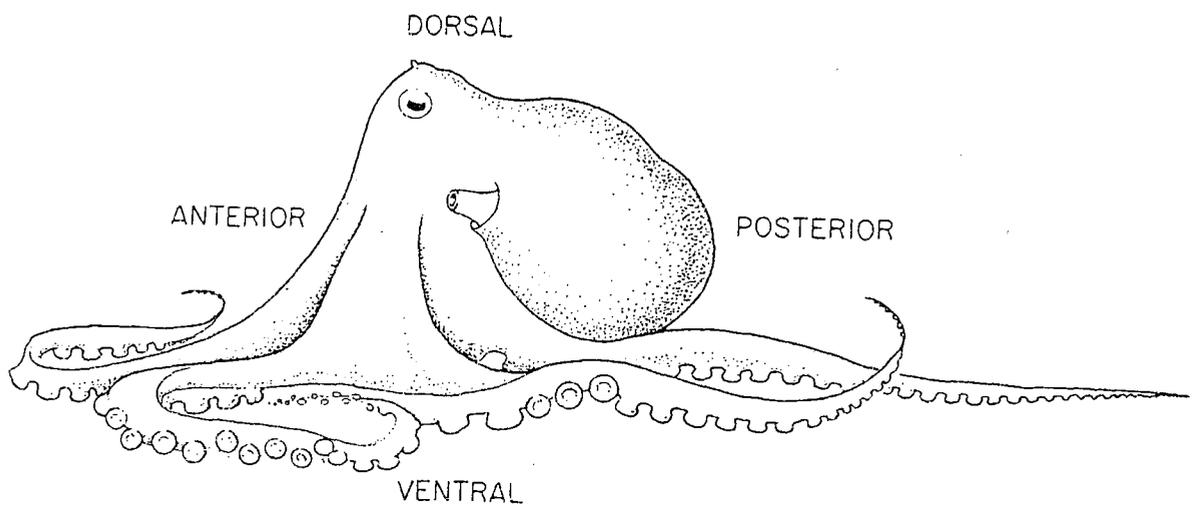


# Skin patterning in *Octopus vulgaris* and its importance for camouflage



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Skin patterning in *Octopus vulgaris*  
and its importance for camouflage

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## ABSTRACT

Camouflage is a method by which animals obtain concealment from other animals by blending in with their environment. Of the cephalopods, the octopods have an extra-ordinary ability to match their surroundings by changing the colour and texture of their skin. Skin patterns of the common octopus, *O.vulgaris*, are built up hierarchically from elements, units and components. Chronic patterns are long-lasting, and are most commonly worn by *Octopus*; acute patterns last only seconds or minutes. Patterns are made up of components. Light and dark (chromatic) components can be distinguished, as well as components related to skin texture, or those related to posture and movement of arms, mantle, funnel, head and eyes. Components combined in series with like components and in parallel with unlike components, comprise whole patterns. At close range, *Octopus* skin is seen to be made up of units; patches surrounded by grooves. Papillae often arise from the centre of patches. Each "chromatic unit" is made up of 3 types of elements. Most superficially lie differently coloured chromatophores; underneath these are two types of reflecting elements, reflecting cells and leucophores. Chromatophores can be expanded or retracted by their radial muscle fibres, which are under nervous control. The nervous control system is hierarchical: the paired anterior and posterior chromatophore lobes send nerves to innervate chromatophores of the head, arms and mantle. These lobes receive fibres from the paired lateral lobes which, in turn, receive fibres from the paired optic nerves, the ultimate controllers of chromatophores. Interestingly, *O.vulgaris* is found to be colour-blind. However, this animal is very capable of discerning differences in brightness, and this information is used to regulate the state of the chromatophores, thereby matching the tone of the surroundings. The reflector cells and leucophores (especially revealed in bright light when chromatophores are contracted) reflect incident wavelengths. The colour-blind *Octopus* is able to perfectly match its surroundings by actively forming a good intensity match using its chromatophores, thereby incidentally revealing a reflecting system that is able to match the background hues. Thus, if *Octopus* takes care of the luminance, the colour takes care of itself resulting in perfect camouflage.

# **1. INTRODUCTION**

## **1.1. Camouflage in marine invertebrates**

Camouflage can be defined as "the art of disguising". It is a method by which animals obtain concealment from other creatures by blending in with their environment. The concealed animal is thus not easily recognised as an animal, but is rather perceived to be a part of the non-living or inedible background.

Camouflage is usually thought of in terms of vision, although several marine invertebrates also practise chemical or tactile camouflage. It can be obtained by fixing or changing colour, shape, texture, chemical secretions as well as behaviour.

In marine invertebrates, camouflage is often used as a defense mechanism. An animal may use camouflage to lower its chances of being noticed and recognised as prey by its predators. However, it is seldom an animal's only means of defense; many invertebrates are also able to run, swim, pinch, bite, or release toxins when they are in danger. Camouflage is also used by predators, allowing them to remain unnoticed until their prey comes within reach. This strategy is especially used by so-called lurking or ambush predators, such as octopods and spider crabs.

Camouflage involves expenditure of time and energy. Species which disguise themselves by attaching various materials onto their body, must be able to find and manipulate these materials. Such animals may need to change their 'dress' when moving to a new environment, or during growth (i.e. crabs). Species which camouflage themselves by changing colour or texture must be able to detect their background in order to activate the appropriate body parts to correctly match their surroundings. Once camouflaged, an animal must remain on the appropriate background or correct its colour and texture when moving to a different area. Movements of camouflaged animals must be characteristic of the environment or be confined to short periods of time, or to the night (Wicksten, 1983).

## **1.2. Cephalopods**

Most cephalopods (e.g. octopods, squids, cuttlefish) have an extraordinary ability to camouflage themselves by changing their skin colour. It is the octopods which are the "chameleons of the sea", being able to perfectly match their surroundings by changing the colour and texture of their skin, as well as completing their disguise with the appropriate posture and movement. A quick systematic review of the cephalopods is not out of place here.

The *Cephalopoda* are a class of the phylum *Mollusca*. This class is entirely marine and is made up of  $\pm 650$  species; fossils represent over 7500 additional species. The cephalopods are basically pelagic, although bottom-crawling occurs in octopods as a secondary adaptation. The cephalopods are carnivorous and most species actively swim in pursuit of prey. Their predacious lifestyle involves various specialisations. The cephalopods possess the most highly organised central nervous system as well as the most sophisticated eyes in the invertebrate world.

The *Cephalopoda* are divided into 3 sub-classes (Barth & Broshears, 1982): the *Ammonoidea*, the *Nautiloidea* and the *Coleoidea*. The first sub-class, with  $\pm 5000$  species, characterised by a coiled external shell with complex septa and sutures, is extinct. The *Nautiloidea*, recognisable by their straight or coiled external shell with simple sutures, are represented by  $\pm 2500$  fossils and only 1 living genus; *Nautilus* (which possesses many slender, suckerless tentacles). All other cephalopods living today belong to the sub-class *Coleoidea*. These are characterised by an internal shell, which may be reduced or absent, and by 8 or 10 appendages bearing suckers.

The *Coleoidea* are divided into 5 orders (Barnes, 1987): the *Belemnoidea*, the *Vampyromorpha*, the *Sepioidea*, the *Teuthoidea* and the *Octopoda*. The first order (*Belemnoidea*), characterised by a chambered internal shell, is extinct. The second order (*Vampyromorpha* or vampire squids) comprises only 1 deep-sea species which has 10 arms (2 of which are minute). The third order (*Sepioidea*) includes cuttlefish and sepiolas, each having 8 arms and 2 tentacles attached to a short or broad, sac-like body. The fourth order (*Teuthoidea*) consists of squids which have an elongate body with 8 arms and 2 tentacles. Species of the last order, the *Octopoda*, are characterised by 8 arms and a globular body without an internal shell.

The aim of this study is to take a closer look at the make-up of camouflage patterns in cephalopods, in particular *Octopus vulgaris*.

## 2. GENERAL BIOLOGY OF THE OCTOPODS

About 150 octopod species have been identified. Octopods have completely lost their ancestral shell, their soft body being globular and bag-like, without fins. Unlike most cephalopods, octopods are bottom dwellers and are typical ambush predators. Although octopods are capable of swimming by water jets, they more frequently crawl about over the rocks around which they live, using jet-propulsion only for escape or long distance swimming. The arms, which are provided with adhesive suction discs, are used to pull the animal along or to anchor it to the substratum as well as for catching prey. Species of *Octopus* usually live in dens located in crevices and holes. The animals make excursions in search of food or lie in wait near the entrances of their retreat. Clams, snails, crustaceans or fish are seized and dragged into the den where they are eaten. The octopus descends upon its prey, enveloping it in its outstretched arm web. Octopods may take several animals back to their den for consumption, all paralysed by a salivary toxin (Barnes, 1987).

### 2.1. *Octopus vulgaris*

The common octopus, *O. vulgaris*, reaches a maximum total length of 3 m. The eight long and very mobile arms carry two rows of suckers (Grzimek, 1973). *O. vulgaris* is best known of the more than one hundred species of *Octopus* actually described. It was known long before 1797, the year in which it was named and described by Lamarck. This species has a world-wide (see figure 1) distribution in tropical, sub-tropical and temperate areas; it is not found in sub-polar or polar regions. It is abundant in the Mediterranean Sea, the eastern Atlantic and in Japanese waters (Mangold, 1983). On a world-wide basis, *O. vulgaris* encounters temperatures between 6 and 33 °C, but it is most often found in waters warmer than 10 °C and cooler than 30 °C. The salinity ranges from about 32 to 40‰.

*O. vulgaris* has been, and still is, extensively used for investigations on the central nervous system. There is already a wealth of information on learning and behaviour. Most of the physiological work of octopods has been done on *O. vulgaris*, as this animal is easy to keep in captivity.

*O. vulgaris* is a truly coastal species, living between the surface and a depth of 100-150 m. Its abundance decreases with depth. The species is well adapted to live in different habitats; it can take advantage of the smallest crevices and holes to hide. *O. vulgaris* is mostly seen to inhabit coral reefs or rocky bottoms, but it may also be abundant over sandy or muddy bottoms or in sea grass. Its capacity to conceal itself on any substrate, by varying colour, skin texture and posture, is challenged by few other cephalopod species.

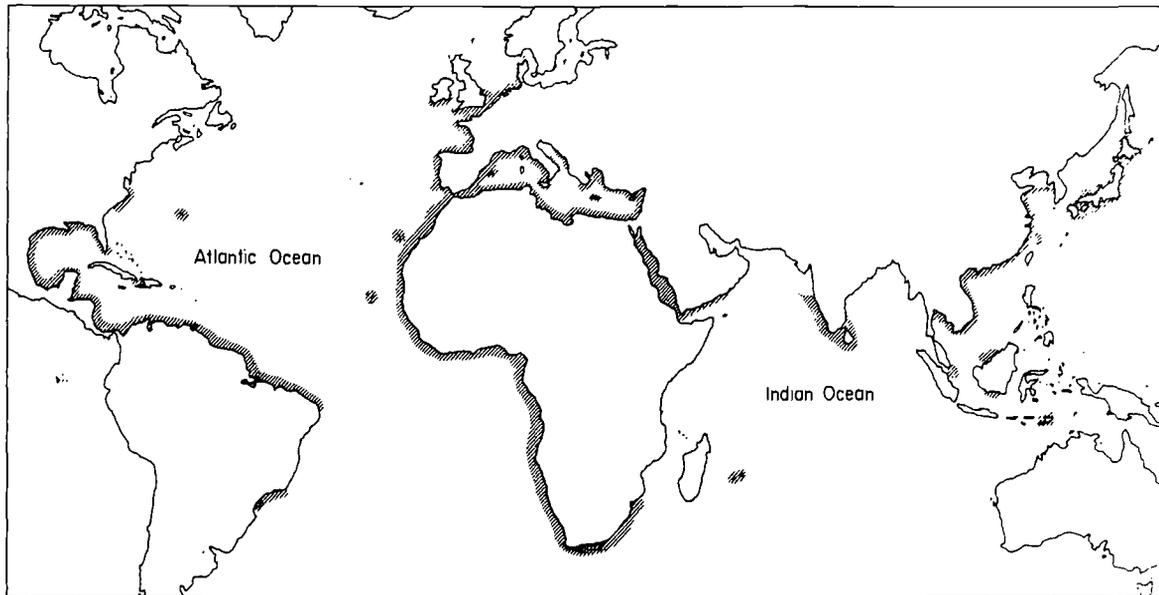


FIGURE 1. World map showing the distribution of the common octopus, *O. vulgaris* (Mangold, 1983).

*O. vulgaris* is truly solitary. Even in the laboratory, if animals have to share a tank, they will try to occupy a home at some distance away from the other inhabitants. This individualistic behaviour is only interrupted during mating and spawning, but the females brood their eggs in isolation. The animals usually remain in their dens, leaving them at dusk for hunting trips to return to them at dawn. Thus, *O. vulgaris* is a typically nocturnal animal.

The life cycle of *O. vulgaris* covers 1 or 2 years. The duration of the embryonic stage depends on temperature; it varies between a little over 20 days to 4 or 5 months. The small hatchlings typically spend several weeks as active predators in the plankton and then settle down to the benthic mode of life at a weight of  $\pm 0.2$  g. The age at settlement may vary between 5 and 13 weeks. Males become sexually mature when smaller in size and younger than females. The size (and age) at spawning depends largely on the combined action of temperature, light and food. Females usually die after the last embryo has hatched, as they do not eat during the brooding period, so that males probably outlive the females.

### 3. SKIN PATTERNING IN OCTOPUS

Clearly, many of the body patterns worn by *Octopus* are for concealment. They succeed in matching the overall reflectance of the immediate environment by representing colour and shape features in it, by obliterative postures, and by disruptive effects in which papillae (small, fleshy projections of the skin), suckers, mantle re-shaping, arm twisting and gross discontinuities of colour and tones destroy the coherence of the body's outline (Packard & Sanders, 1969). Any patterns worn by *Octopus* which are clearly visible, are potentially available for signalling (i.e. for intra- or inter-specific communication).

Skin patterns of *Octopus* are built up hierarchically from elements, units and components (Packard & Sanders, 1971). The dorsal surface of *O.vulgaris* has been studied in detail, and, unless otherwise stated, the following account of the make-up of skin patterns refers to this species.

#### 3.1. Patterns

The appearance of a particular octopus at any one moment in time is called the body pattern. Different patterns can be perceived and recognised by an observer, but classifying them is a difficult task. Principally speaking, "there are as many patterns as can be recognised by the classifier" (Packard & Sanders, 1971). There are several patterns that serve to conceal the animal, as well as different patterns for display.

The patterns most commonly worn by *Octopus* are called "chronic patterns" and these are sustained for long periods of time (hours or days). Not only are these the commonest patterns worn by *Octopus* while at rest, walking or swimming, but one pattern will be characteristic of an individual over a number of days. The animal returns to it after acute variations in intensity, or after assuming a completely different pattern for a short period of time. "Acute patterns" are those patterns lasting only seconds or minutes.

Chronic patterns, also called "chronic general mottles" or "uniform phases" consist of fine mottling of all visible areas of the skin; papillae are present but low. The general colour and tone achieved is the same for all exposed areas of the body, though may sometimes be in a different phase on one side of the body from that on the other ("unilateral"). Overall colours vary greatly, from reds to browns of different hues. Components such as the eye-bar, the arm-bars, the arm spots and the hood are often superimposed upon the general mottle, especially at low intensities.

Acute patterns are short-lasting, but at low intensities they may also be long-lasting. There are several "acute resemblance patterns" that are worn by *Octopus* to achieve a careful match of the body with its surroundings. This is achieved by all means available to the animal: mottling, dappling, blotching; eye masking, disruptive contouring (through differential raising of papillae and projection of suckers); obliterative flattening or bunching up of the body; adoption of adventitious material (sand, pebbles, shells) that are held up against the body, and very slow movement or a motionless posture (Packard & Sanders, 1971).

"Components" are, by definition, the parts that go to make up the whole, and patterns can be described in terms of the relative positions (arrangements) and intensities (values) of components regularly present.

Packard and Hochberg (1977) have formulated the following general principles of patterning, for *Octopus*:

- 1) Components vary in the extent of their expression, from barely perceptible to fully expressed.
- 2) Single components (e.g. a white spot, a raised papillae) do not appear in isolation, but along with other components of the same category (i.e. in series). When such serially repeating components are expressed with less than maximal intensity, a proximo-distal gradient of intensity can be observed at any one moment (e.g. bars further away from the head, or nearer to the arm tips, are faintest).

There are 2 exceptions to this principle:

- i) Components may express themselves unilaterally, with little or no sign of the same components on the other side of the body.
  - ii) Some components are by nature single components and can be expressed in apparent isolation from others (i.e. eye-bar, eye-spot, eye-ring).
- 3) Different components combine together (i.e. in parallel) to give distinct patterns. Patterns are made up of a combination of different sets of components, including postural components (shape of body, position of arms) that go together with a certain appearance of the skin.
  - 4) Patterns are reflections of the whole behaviour of the animal. Put in another way; body patterns are themselves components of particular behaviour sequences.

Thus, components combined in series with like components and in parallel with unlike ones comprise whole patterns.

A given pattern, which may vary in intensity of expression, reflects a specific behaviour and can be evoked under given circumstances. Coloured (light or dark) components, together with changes in skin texture, body shape and movement, combine as complete patterns. Patterns can be seen to grade into each other.

Individual octopuses - and the same is true of other cephalopods - show patterns that appear to be quite stereotyped. Time and again the same pattern, with individual components involving the same set of skin patches each time, will be exhibited under the same set of conditions. However, the combinations of components both within individuals and between individuals are not entirely fixed.

### 3.2. Components

We have already seen that patterns are made up of components. Components are, in turn, built up of units and elements. Normally, components appear as complete features suggesting that they are anatomically constant, each presumably under the control of only a few neurones in the higher motor centres (i.e. the optic or chromatophore lobes - see section V).

A single component does not in itself comprise a given body pattern, though it may be a dominant feature of a pattern. Such are the white spots on the front of the head. These frontal white spots have an immediate attention-focusing function, unlike other components which are perceived as part of the whole. By concentrating attention onto themselves, the white spots protect the wearer from discovery by a predator's wandering gaze.

Packard & Sanders (1971) give a fairly complete list of components for *O. vulgaris*. The components may be expressed in a variety of combinations, each combination classifiable as a separate whole pattern. Commonly, certain components go together (e.g. widened pupil, white spots and dermal papillae) whilst others are mutually exclusive (e.g. stretched arms and upturned arms).

Undoubtedly, the great variety of body patterns seen in *O. vulgaris* is not so much due to different combinations of components as to differences in the intensity of expression of each component. This is especially true for cryptic patterns, which are capable of granting the octopus visual conformity with almost any background.

In *Octopus*, several "chromatic components" can be recognised and these are divided into 2 types: light and dark components. There are also various different components related to skin texture as well as to the posture and movement of the arms, mantle, funnel, head and eyes. Locomotory movements have not been analysed.

### 3.3. Units

Looking more closely at *Octopus* skin, we can see that it has the appearance of a loosely woven network of which the strands are usually darker than the rest of the skin. The individual "cells" of the net may be circular, oval or polygonal, varying in diameter from less than 1 mm to several millimetres. They have a characteristic upper size - equal to the mesh size of the net - and in some species there are also many cells of small diameter filling in the nodes between the main (major) cells. Packard & Sanders (1971) termed the lines of the net as a system of "grooves" and the areas which they bound as "patches". A skin patch and its surrounding groove is one "unit".

The network is anatomically fixed. The number of major patches over any one region of the body remains constant from early on in ontogeny, the patches growing in size as the animal grows (though increasing numbers of smaller patches become distinguishable as the main network stretches with growth).

The skin of a groove dips inward; the depth of the infolding, and inversely the width of the groove, varies much in life. In relaxed skin, the grooves are shallow and broad. When the muscles of the skin are contracted as well as in older animals, the grooves are deep and narrow. The appearance of the skin at any one moment depends to a large extent on the way in which the individual parts of the network are disposed. Individual patches, or groups of a few patches (not necessarily making up a component), may be used singly to produce visual effects.

Many of the units, especially the larger ones, may be seen to have tributary grooves running into the patch from the surrounding groove. These sometimes form a short spiral - indicating that the patch contributes to the formation of a papilla. At points on the skin where major papillae arise, the patches are arranged concentrically with the smallest patches at the centre, forming the peak of the papilla. The location of papillae does not change and a given papilla will always have the same patch at its peak. Papillae do vary in height and some of the larger ones (e.g. the 4 large mantle papillae, the long papillae above the eyes) carry smaller second- and third-order papillae on their lower slopes during full expression.

*Octopus* has 3 types of elements which are employed in colour patterning; being too small to produce visual effects in isolation, these elements act in combination. First, details of each of these elements lying in the dermis will be given before expanding on the make-up of the patch and groove structure (also called "chromatic unit") of *Octopus* in chapter 4. The functioning of the different elements in the chromatic unit will be discussed in chapter 7.

### 3.4. Elements

*Octopus* can match its background colour with the help of three elements present in the skin. Most superficially are the "chromatophores" (colour-bearers), followed by two types of reflecting elements, the "reflector cells" and, deepest in the dermis, the "leucophores".

#### 3.4.1. CHROMATOPHORES

In the beginning of the 19<sup>th</sup> century, people began to study the characteristics and mechanism of colour-changes in cephalopods. At first, it was believed that the colour was produced by some coloured liquid but soon it was discovered that the coloured spots were stable and that they did not change their place but only their form. Sangiovanni (1891) was the first to recognise that these colour-changes were the effect of some special little organs he named "chromophores". This was later changed into chromatophore, meaning colour-bearer. The general features of cephalopod chromatophores are now understood in considerable detail (Cloney & Florey, 1968; Florey, 1969; Mirow, 1971a).

The cephalopod chromatophore organ consists of 5 basic features, the first 4 are diagrammatically illustrated in figure 2:

- 1) The cytoelastic sacculus, containing pigment granules (also called chromatophore or pigment container).
- 2) Radial muscle fibres, attached to the margins of the cytoelastic sacculus.
- 3) Neuronal processes (axons), associated with each muscle fibre.
- 4) Glial cells, accompanying each axon (also known as Schwann cells)
- 5) Sheath cells, which surround the chromatophore and its associated nerves and muscle fibres.

The chromatophore organs are not static units, but they expand and retract according to the state of the radial muscle fibres attached to them. When the muscle fibres relax, the chromatophore assumes its retracted shape whereby the surface of the chromatophore becomes extensively folded. The primary infoldings of the chromatophore surface are anchored to the cytoelastic sacculus at points called focal haptosomes.

The pigment granules are confined within the cytoelastic sacculus throughout the cycle of expansion and retraction of the chromatophore. The periphery of the sacculus is attached to the plasmalemma along the equatorial part of the chromatophore, opposite the areas of attachment of the radial muscle fibres by zonular haptosomes. Within these zones, much of the tension generated by the radial muscle fibres is transmitted to effect chromatophore expansion.

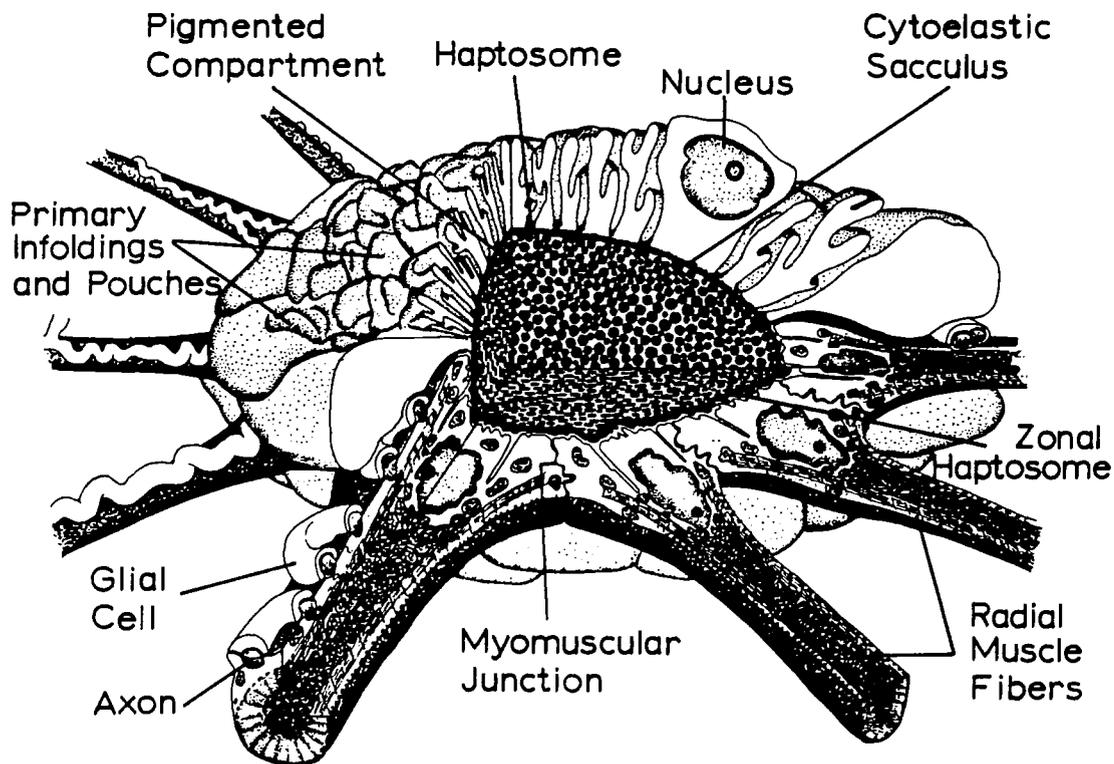


FIGURE 2. Cut-away illustration of a retracted squid chromatophore organ showing the arrangement of nerve fibres, radial muscle fibres, and the folding of the plasmalemma around the pigment containing compartment. Only a few muscle fibres are shown; sheath cells have been omitted (Cloney & Florey, 1968).

There are 10-30 radial muscle fibres per chromatophore. These fibres are regularly, obliquely striated and are single, unbranched cells with their nuclei situated at the periphery of the chromatophore. The central core of each muscle fibre is densely packed with mitochondria. It was first believed that the muscle fibres operated together, forming a syncytial system surrounding a chromatophore cell. Bozler (1931) produced evidence against the syncytial nature of the chromatophore muscles, providing evidence that muscle fibres can be individually controlled by nerve fibres and can function as independent units, even though they form close junctions with their neighbours.

Also, it was believed that the distal end of the muscle fibres was situated some distance away in the connective tissue, possibly attached to either strands of connective tissue or skin muscles. Later, microscopic evidence showed that chromatophore muscles do not end blindly, but interconnect individual chromatophores (Froesch-Gaetzi & Froesch, 1977).

Muscle fibres are innervated by one or more nerve fibres. Each muscle fibre is innervated by a separate nerve branch or axon. The axon bifurcates about half way along the length of the muscle fibre; one branch travels distal and the other proximal. In its course along the length of a muscle fibre the axon follows a serpentine pathway, thus permitting the muscle to change in length without imposing excessive tension on the nerve or on its junctions with the muscle fibre.

The axons are always accompanied by cellular processes of glial cells. The entire chromatophore, part of its muscles and the nerves to these muscles are enveloped by sheath cells.

### Expansion and retraction of the chromatophore

When the radial muscle fibres contract, the retracted chromatophore increases up to seven times in diameter (Florey, 1966) and assumes the shape of a flat disc. The primary infoldings straighten out and the cytoelastic sacculus (with its cytoplasm and pigment granules) is forced into a thin layer. During the course of expansion, the equatorial regions of the chromatophore expand into regions previously occupied by muscle fibres and sheath cells. At the same time, the sheath cells move into the space vacated by the spreading, flattening chromatophore. The energy for the entire expansion-retraction cycle appears to be derived from the mitochondria present in the contracting muscle fibres.

Retraction begins with the relaxation of the muscle fibres. The elastic properties of the cytoelastic sacculus return the pigment mass to its resting, lenticular shape, as well as fold the plasmalemma and reorient the sheath cells.

The chromatophore organs are located in the dermis, in between layers of connective tissue. Mirow (1972a) pointed out that, in *Loligo paelii* and *L.opalescens*, all cells of the dermis are orientated with their long axes parallel to the surface of the skin. This orientation presumably allows for chromatophore movement to occur without distortion of other elements present in the skin.

### Development of chromatophores (morphogenesis)

During the embryonal period of *O.vulgaris*, chromatophores occur in the mesoderm only; a central cell arises at an early stage and is later surrounded by independently formed radial fibre cells (Fioroni, 1970). At hatching, *Octopus* has a larval set of about 70 stable, undifferentiated chromatophores; the majority of these is present on the ventral surface of the body.

*O. vulgaris* has a maximum of 8 tegumental (founder) chromatophores on the dorsal mantle surface at hatching (Fioroni, 1965); a forward pair (sometimes 3 or 4), at the boundary between mantle and head, and a posterior pair (rarely 3 or 4). The small number of tegumental chromatophores is characteristic for the hatchling, as is the absence of reflector cells (formed at the end of the planktonic phase).

The octopus settles out of the plankton at 4-6 weeks of age. At this time, the rate of chromatophore genesis of the dorsal mantle field rapidly increases, overtaking the rate of recruitment of the ventral field. This results in three differences between dorsal chromatophores and those lying ventrally: the former are smaller than the latter, they are present in higher local densities, and dorsally there are more members of different classes than on the ventral side of the animal (Packard, 1985).

Even when the tiny individual has grown 10 000 fold in size (from  $\pm 3$  mg to 30 g) the founder chromatophores can still be discerned (unaltered in size, shape and relative position), embedded amongst several thousands of chromatophores that have arisen in the intervening space and time. There seems to be a spacing principle at work, as chromatophore arrangements are always patterned, never random. In *Octopus*, nearly half of the chromatophores arise in the neighbourhood of established or founder chromatophores, although there is a minimum 'nearest neighbour distance' of  $\pm 100$   $\mu\text{m}$ . This arrangement agrees with Meinhardt and Gierer's (1974) "theory of lateral inhibition" which involves activator and inhibitor substances, each with their own diffusion ranges, which disperse from established chromatophores.

Packard (1982) has developed nine "rules" for the development of the morphological array (morphogenesis) of chromatophore clusters for *Octopus*, but these appear to be general rules for cephalopods:

- 1) General recruitment.  
Recruitment of chromatophores into the mantle, head and arm fields occurs from germinal cells spread throughout each expanding field.
- 2) Field effects.  
The rate of recruitment varies from one part of the field to another, according to proximo-distal and dorso-ventral gradients established early in ontogeny, and to local conditions that alter recruitment.
- 3) Field subdivision.  
As each field expands, sub-fields arise within it.
- 4) Position and size constancy.  
Once established, a chromatophore does not alter its position in the field nor are there appreciable changes in resting shape.

- 5) **Mutual relationship.**  
Established chromatophores influence the position, size and rate of differentiation of further chromatophores arising in their neighbourhood through processes of lateral inhibition, which result in chromatophores being spaced out relative to each other.
- 6) **Size hierarchy and age.**  
Chromatophores can be ranked according to size. The size hierarchy (based on resting diameters) in a cluster reflects the order in which the chromatophores were formed. The largest chromatophores are the earliest; the smallest are the latest.
- 7) **Age and colour.**  
When chromatophores first arise, they are pale (usually yellow or orange in colour) and become progressively darker with age.
- 8) **Colour and degree of expansion.**  
The depth of a chromatophore's colour is inversely proportional to its degree of physiological expansion.
- 9) **Separate responses.**  
Chromatophores of a given size and colour class are able to expand or retract independently from other size and colour classes (i.e. each age class can have its own innervation and muscle connections).

As far as is known, these nine rules continue to operate throughout ontogeny. Figure 3 illustrates some of the rules of morphogenesis. By the end of a year, the adult *Octopus* possesses 1-2 million chromatophores, most of them on the dorsal surface of the body (between 100-200 per square mm).

As said, the chromatophores of *O. vulgaris* are by no means arranged haphazardly; the different types are arranged amongst one another and sometimes form quite regular two-dimensional arrays. Whatever the mechanism for the non-random distribution of the chromatophores, it is presumably advantageous for camouflage.

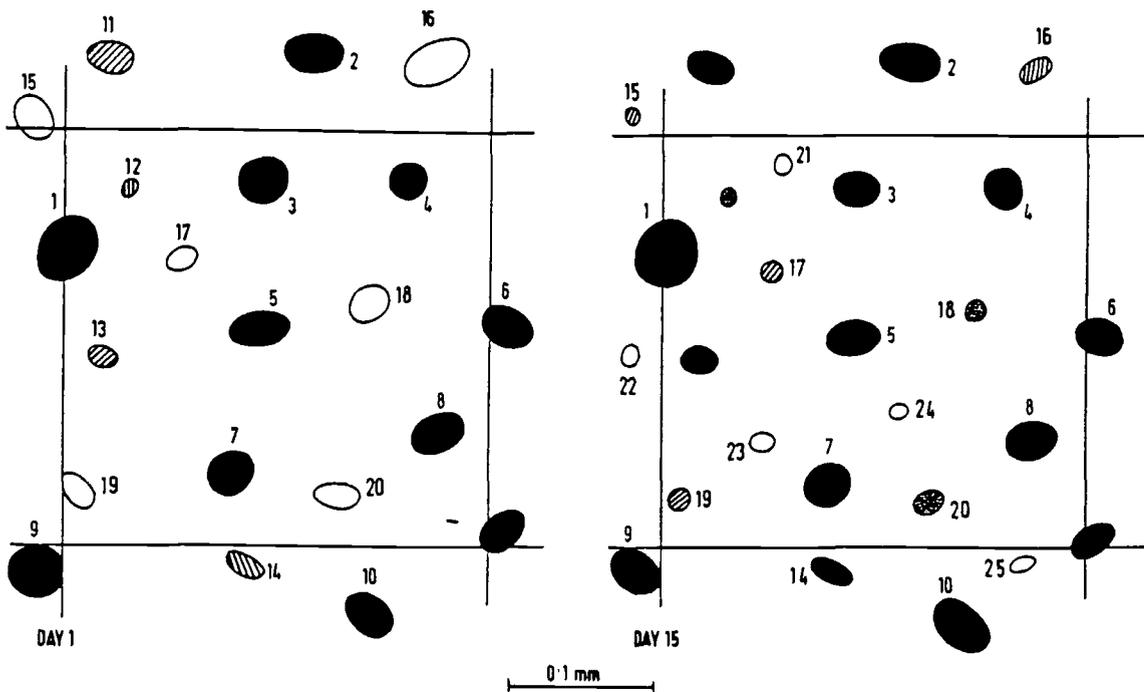


FIGURE 3. Diagram illustrating the principles of morphogenesis in the dorsal mantle surface of *O. vulgaris*. Positions, shapes, sizes, and colour of individual chromatophores are shown on Day 1 (left) and Day 15 (right). Chromatophores are numbered for ease of identification: mature chromatophores (melanophores) are shown black, the depth of pigmentation of other chromatophores is indicated by shading, yellow ones are unshaded. All chromatophores are in retracted condition, except the yellow ones on Day 1 (Packard, 1985).

### Types of chromatophores

The chromatophores present in the skin of octopus have different hues, ranging from yellow, through orange, red and brown to black (the latter are also called melanophores). The members of the chromatophoral array lie in staggered positions relative to each other and in successive layers of the dermis. The lightest (pale yellow) chromatophores lie deepest in the dermis, followed by the orange ones, the red ones, the brown ones and most superficially the melanophores. The former have small pigment granules whilst the latter have large granules. Using microprobes, Froesch & Packard (1979) analysed the pigment granules which revealed rising amounts of calcium, nickel and sulphur as the size of the granules increased (associated with a change in colour from yellow to, finally, black). This is regarded as evidence that the differently coloured chromatophores are not different types of chromatophores as previously thought, but that they are to be regarded as members of a continual series, caught at successive stages of differentiation.

### 3.4.2. REFLECTOR CELLS AND IRIDOPHORES

As we have seen, the skin of *Octopus* and of other cephalopods contains several layers of chromatophores. Underneath these lie two different types of reflecting elements; iridophores and leucophores. Much less is known of these structures than of the colour-bearing chromatophores.

There are, strictly speaking, 2 kinds of iridophores found in cephalopods. *Octopus* possesses only one kind of iridophore, the so-called reflector cells (see figure 4; description follows), whilst other cephalopods possess reflector cells and true iridophores. Both types are collectively known as iridophores (occasionally called iridiophores or iridocytes). In *Octopus*, aggregates of tiny reflector cells within the dermal connective tissue form a reflecting stratum beneath the chromatophore organs, throughout the integument. The third element involved in skin patterning, the leucophores, are situated beneath this layer.

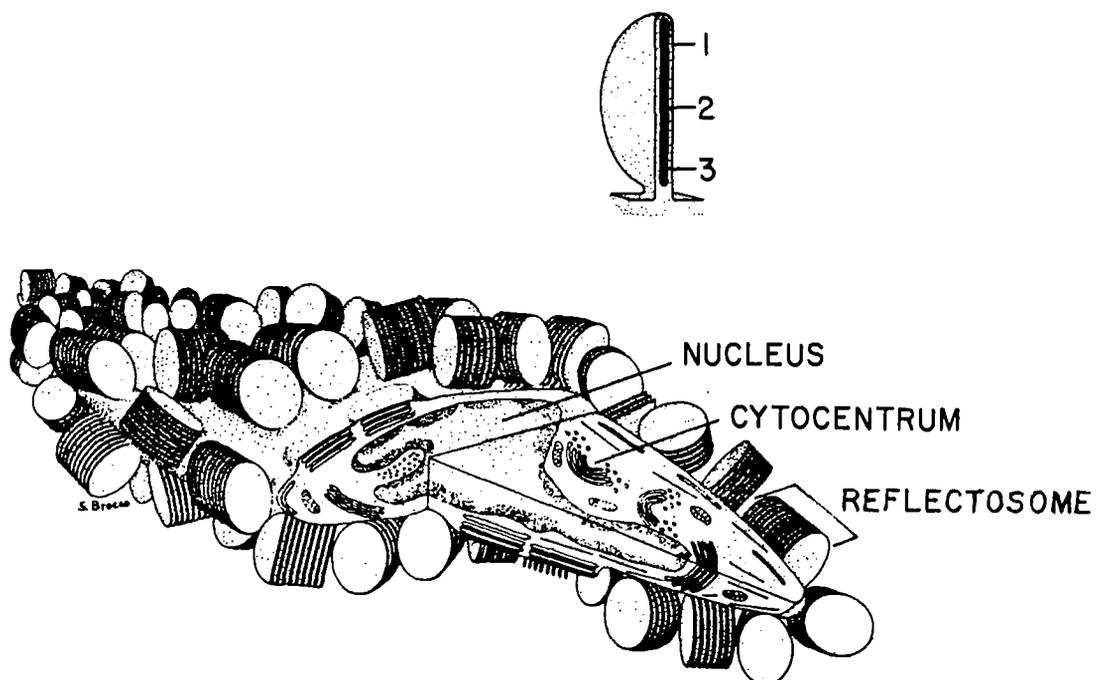


FIGURE 4. Diagrammatic model of a reflector cell of *O.vulgaris*, showing the flattened, ellipsoidal, nucleated cell with its peripheral zone of radiating cytoplasmic processes, the reflecting lamellae. The latter are arranged into functional light reflecting groups, the reflectosomes. Each discoidal reflecting platelet is a tripartite structure (inset) consisting of a central discoidal, reflecting platelet (3) surrounded by a thin layer of cytoplasm (2) and by the plasmalemma (1) (Brocco & Cloney, 1980).

Taking a closer look at the structure of a reflector cell of the giant Pacific octopus, *O.dofleini*, we see that each cell is nucleated, flattened and ellipsoidal in shape (Brocco & Cloney, 1980). A reflector cell bears thousands of peripherally radiating, discoidal cytoplasmic processes called reflecting lamellae. Each lamella is a tripartite structure made up of a central, discoidal reflecting platelet surrounded by a thin layer of cytoplasm and by the plasmalemma (see figure 4). The lamellae, having platelets variable in diameter, are arranged into functional groups called reflectosomes. Each reflectosome consists of 2 to 32 lamellae orientated with their flattened surfaces in register, similar to a stack of coins. Adjacent reflecting lamellae in a reflectosome are separated from each other by an intracellular gap, the interlamellar space.

The reflectosomes are randomly orientated about the axis that intersects the cell body. Consequently, the planes of the different reflectosomes are not parallel. Brocco & Cloney (1980) estimate that each reflector cell bears 2000-3000 reflecting lamellae. Based on an average of 11 lamellae per reflectosome, a typical cell would bear 180-270 reflectosomes.

The observed organisation of a reflectosome is compatible with its role as a "quarter-wave thin-film interference device" (Denton & Land, 1971). The alternating reflecting lamellae and interlamellar spaces constitute layers of high and low refractive indices. In theory, the highest reflectivity at a given wavelength  $\lambda_0$ , together with the widest wave band of high reflectivity, is obtained when such alternate layers each have an optical thickness of  $1/4\lambda_0$ .

The reflector cells (or true iridophores) of cephalopods come close to such theoretically "ideal" reflectors. The platelets and the cytoplasm layers within a reflectosome are very thin (between about 70 nm and 120 nm); their optical thickness (actual thickness X refractive index) closely approximates that of ideal quarter-wavelength reflectors. In white light, they will produce colours by constructive interference. The platelets of cephalopods have a refractive index of  $\pm 1.56$ ; the calculated best reflected wavelength is blue-green which coincides with colours observed to be best reflected in vivo.

The blues and greens reflected by these cells are particularly well suited for matching the sub-tidal marine environments, where the predominant wavelengths are in the 460-490 nm range. In *Octopus* skin they are especially important for producing these hues, but as the reflector cells are orientated in all planes they can also produce white broad-band reflection.

The reflector cells of *O.dofleini* are in the same relative positions as true iridophores in studied species of squid and cuttlefish (Mirow, 1972b; Brocco & Cloney, 1980).

As mentioned before, squids have reflector cells as well as true iridophores; the reflector cells are present in the iris, eyelids and sclera as well as in the lining of the inc sac, whilst true iridophores are found in the skin (Hanlon, 1982). These non-pigmented cells reflect, diffract and scatter incident light.

True iridophores can be distinguished from reflector cells by the orientation of their platelets; these are orientated on edge, relative to the surface of the skin, instead of having their broad side facing the integument (as in reflector cells). In true iridophores, the individual platelets are called iridosomal platelets and are separated from each other by interplatelet spaces; the arranged functional unit of platelets and spaces is called an iridosome.

Cephalopod platelets are flexible and were first thought to be made up of guanine or chitin. The exact substance is still unknown, but it seems to be a sulphur rich protein (Brocco 1977).

### 3.4.3. LEUCOPHORES

*Octopus* lacks true iridophores, having only reflector cells in the dermis. However, it has a second type of reflecting element which is not found in squids; the leucophore. Little is known about leucophores and they have only been described for *Octopus* species. Leucophores were first described by Packard & Sanders (1971) as being irregularly shaped and containing a creamy guanine-like substance. Later, the structure of leucophores was described in more detail by Brocco (1975; 1981), for *O.dofleini*, and by Froesch & Messenger (1978), for *O.vulgaris*.

The cell bodies of leucophores and reflecting cells are about the same size. At higher magnification, each leucophore is seen to be a complex, much branched, uninucleate cell with the outline of an elongate, compressed ovoid. The leucophores lie with their broadest plane approximately parallel to the surface of the skin. One to two thousand ovoid reflecting organelles (called leucosomes), within narrow club-like cytoplasmic stalks, protrude from all over the surface of the leucophore. In *O.dofleini*, Brocco (1975) estimates that there may be 20 000 leucosomes per leucophore.

White material is present in the leucosomes; this is not homogeneously spread, the centre being much denser than the periphery. The nature of the material in the leucosomes is unknown but it seems unlikely to be guanine, for it is non-crystalline.

Leucophores are found in high concentrations in the anatomically fixed white components of the skin (e.g. arm spots, mantle white spots), together with high numbers of reflecting cells. White spots have already been mentioned as being effective reflectors of incident light and important for producing disruptive effects during camouflage.

Not only are the leucophores of *Octopus* present in white spots, they are also situated at the centres of patches, at the base of large papillae and concentrated at the tips of papillae. Unlike reflecting cells or true iridophores, leucophores do not contribute to structural colours by diffraction but only reflect light; they thus appear chalky white in incident white light and dark in transmitted light. In *O. vulgaris*, leucophores have been shown to be good reflectors of white light. We may therefore assume that they are important in enabling the skin of *Octopus* to reflect whatever incident wavelengths predominate in its environment. Messenger (1974) demonstrated that the tips of papillae can reflect blue, green or red light.

Thus, leucophores act as broad-band reflectors, usually appearing white but if the band width of the light reaching them is more restricted they will reflect that narrower band. Such an arrangement in the skin can "automatically" provide effective camouflage (crypsis), without the bearer having to activate different elements in the skin. The intensity of the white light (or other reflected wavelength) is controlled by the overlying chromatophore layer.

#### 4. THE CHROMATIC UNIT

After a detailed account on the 3 different types of elements present in the skin of *Octopus*, we return to the patch and groove structure (also called a chromatic unit), taking a closer look at its make-up.

The elements employed in colour patterning (chromatophores, reflector cells and leucophores) are by no means randomly distributed in the dermis. The concentration of chromatophores in grooves is less (about half) than over the patches. The characteristically dark appearance of the network of grooves is due to a combination of effects: there are relatively few reflector cells present in the grooves, chromatophores tend to be tonically expanded, and infolding piles up chromatophores normal to the surface when the skin is relaxed (Packard & Sanders, 1971).

The patches are, by contrast, underlain by large numbers of reflector cells that give them a pale appearance in reflected light. The additional white reflecting leucophores are clustered centrally in most patches and some tens of leucophores aggregate here to form a sponge-like reticulum. There are gaps ("sponge holes") between individual leucophores and it does not seem to be coincidence that several dark-red or black chromatophores are situated vertically above these holes. This regularity implies that there is interaction between leucophores and chromatophores during morphogenesis, but whether the final arrangement has any significance for camouflage is not yet clear. By having melanophores over these holes, the animal perhaps maximises the reflecting area it can mobilise in bright light, when the melanophores are fully retracted (Froesch & Messenger, 1978). In *O. vulgaris*, the patches are typically elevated above their surrounding grooves by a complex skin musculature, so that the animal can effectively increase its reflecting surface (Sanders & Young, 1974).

Recently, a physiologically discrete population of "brown spots" was found in *O. vulgaris* (Andrews, Packard & Tansey 1982). These spots are distributed over the dorsal skin of the head, mantle and arms and correspond mainly with the distribution of white spots and streaks; they appear to act as a screen for the patches making up white spots. The brown spots lie over most, but not all of the patches in white spot areas and are composed of chromatophores concentrated near the centre of a patch. These chromatophores are smaller than the surrounding ones and are not the only screening chromatophores in the area.

The papillae of *Octopus* skin often appear white or green because of the reflector cells that are concentrated within them. Because leucophores are sited centrally in the chromatic unit (as are the papillae tips) they form conspicuous white tips to erect papillae, which are important in producing cryptic colour effects.

As we recall, units consist of groupings of elements in the skin. The equivalent of the *Octopus* patch and groove chromatic unit, which contains hundreds of chromatophores, reflector cells and many leucophores in an area little more than 1 square millimetre, is not immediately apparent in other cephalopods (Packard, 1982). Variations in the arrangement of the patches between one species of *Octopus* and another have been tabled in Packard & Hochberg (1977).

In *Eledone cirrhosa* chromatophores and leucophores are organised into "chromatic units", but these are not clearly morphologically limited by grooves as in *O.vulgaris* (Boyle & Dubas, 1981).

Squid chromatophores are almost an order of magnitude larger than *Octopus* ones, with local densities of  $\pm 10$  per square millimetre compared to 100 to 200 in *Octopus*. However, in loliginid squids the dark chromatophores (brown and red) on the dorsal mantle and elsewhere are arranged as a series of continuous and intersecting circles 1-2 mm across, each circle with a single large dark chromatophore at its centre and with pale (yellow) ones interspersed. The circles have similar dimensions to the patches of *Octopus*, and, like the patches, they are (in many parts of the body) underlain by conspicuous accumulations of reflecting material (Hanlon, 1982). Each circle is a morphological unit, having the same status as the patch and surrounding groove of *Octopus*.

Thus, each morphological unit contains all the elements that contribute to patterning in that part of the skin.

## 5. NERVOUS SYSTEM

The patterns worn by *Octopus* are under nervous control. Chromatophores are surrounded by radially arranged muscle fibres, which are innervated by several different nerves.

### 5.1. Motor units

The term "motor unit" refers to a motor neurone and the muscle fibres it innervates. Chromatophore motor units of cephalopods seem to have the following characteristics:

- 1) A single chromatophore and individual muscle fibres can be part of several motor units. In other words; axons contained in several dermal nerves converge to innervate the same chromatophores and individual chromatophore muscles are innervated by several nerve processes.
- 2) A single axon innervates a large number of chromatophores (and within a single chromatophore, several muscle fibres), not all clustered together but scattered over a large part of the chromatophore motor field of the dermal nerve considered. The individual axons branch repeatedly to innervate the radial muscle fibres of chromatophores scattered over several millimetres; neuromuscular contact seems to be made "en passant". Among the chromatophores, axons are found either running singly or in small bundles, often accompanied by sheath cells.

Multiple innervation of chromatophores means that the same chromatophore, under the control of several different nerve fibres, can participate differently in different patterns. In *E.cirrhosa*, the distribution of the motor units coincides with the distribution of mottles shown during normal patterning (Dubas, 1987). Thus, motor units are also units of visible patterning.

### 5.2. Electrical stimulation

Chromatophores alone can be made to expand by using electrical stimulation, causing little or no change in the shape of the skin. The pattern of response to electrical stimulation varies in different parts of the skin (see figure 5), but there are a number of consistent features. One is that many chromatophores expand over a definite region of the skin; usually there is quite a sharp boundary between these and other chromatophores not involved in the response.

The second feature is that movement of the stimulating electrode within the area of responding chromatophores does rather little to change the pattern of response (though additional groups of chromatophores near to the electrode may be recruited whilst others far away drop out). However, as soon as the stimulating electrode crosses the boundary between responding and non-responding chromatophores, the pattern of expansion may change abruptly. So far, only the melanophores have been studied in this way (Packard & Hochberg, 1977).

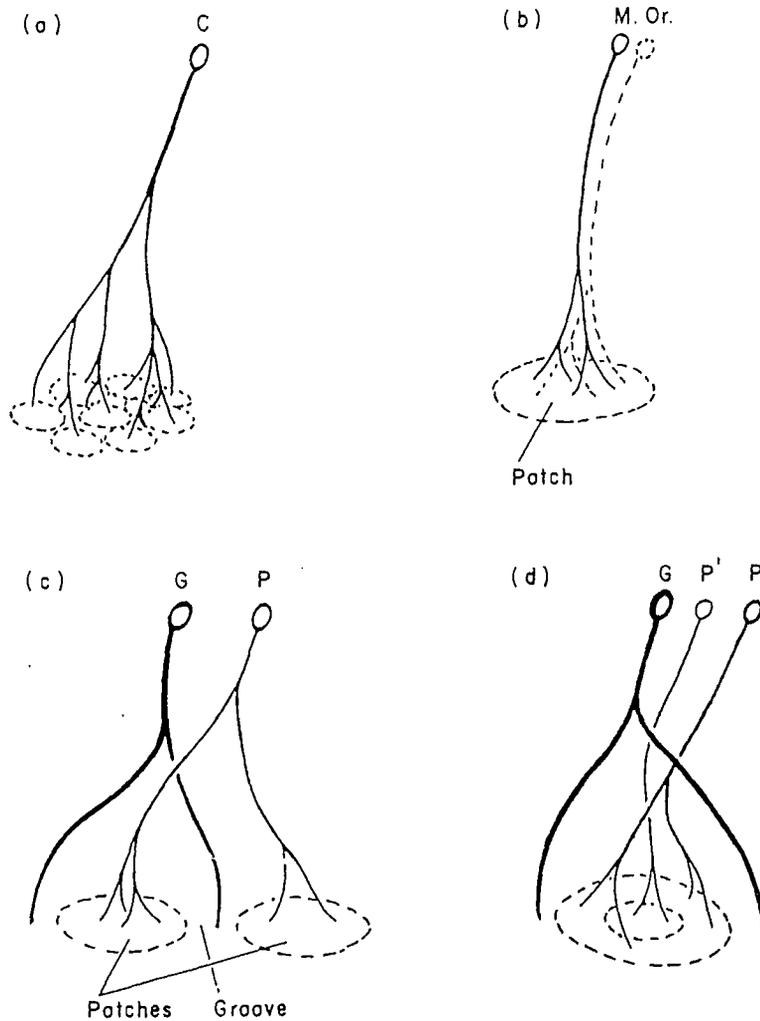


FIGURE 5. Various manners of innervation indicated by electrical stimulation of the skin:  
 (a) A motor unit covering several patches and grooves constituting a whole component (C).  
 (b) Melanophores and orange-red chromatophores within a patch separately innervated by fibres M and Or.  
 (c) Grooves and patches separately innervated by fibres G and P.  
 (d) Differentiation within a patch; chromatophores in the central region are innervated by a separate fibre P'.  
 (Packard & Hochberg, 1977).

Florey (1969) states that in the squid, orange chromatophores are separately innervated from melanophores, and the same may also be true for *Octopus*. It turns out that the sets of chromatophores that do expand - usually hundreds at a time - follow the patch and groove structure of the skin and form parts of the dark components seen under natural conditions.

In *E.cirrhusa*, individual muscle fibres respond to single electrical impulses with twitch-like contractions that do not facilitate with repetition, but summate to a smooth tetanus at about 10-15 Hz. At tetanic frequency, the degree of expansion of single chromatophores is always maximal. However, the number of expanded chromatophores can be graded by variations of either stimulus voltage or frequency (Dubas & Boyle, 1985).

### 5.3. Projection of nerves on the skin surface

By cutting the brain nerves of *O.vulgaris*, Froesch (1973) was able to reveal the projection of the motor chromatophore fibres on the body surface. His study revealed that there are ten nerves which leave the brain, which contain chromatophore fibres. Their areas of colour control are well defined and do not overlap (see figure 6). The largest area, the mantle, is supplied by the pallial nerve and the smallest area, the iris, by the median superior ophthalmic nerve. No nerve cut affected the chromatophores of the funnel; it therefore remains to be discovered whether these chromatophores are under nervous control or not.

In *O.vulgaris*, the capacity to regulate patterns of colours (and skin papillae) on the dorsal surface can recover after interrupting the pallial nerve by either crushing or cutting it (Sanders & Young, 1974). About 40 to 60 days after denervation, spontaneous waves of colour due to the hyper-excitability of the chromatophore muscles cease and the skin begins to change colour in phase with that of the intact side. Specific patches of colour are seen to recover in the precise positions they occupied before operation. Different patterns can again be produced after regeneration, as before, according to different behavioural situations and backgrounds, indicating that nerve fibres can re-make their original connections. Recovery of patterning is not always complete, sometimes it occurs over only part of the dorsal body surface, especially when the nerve has been sectioned.

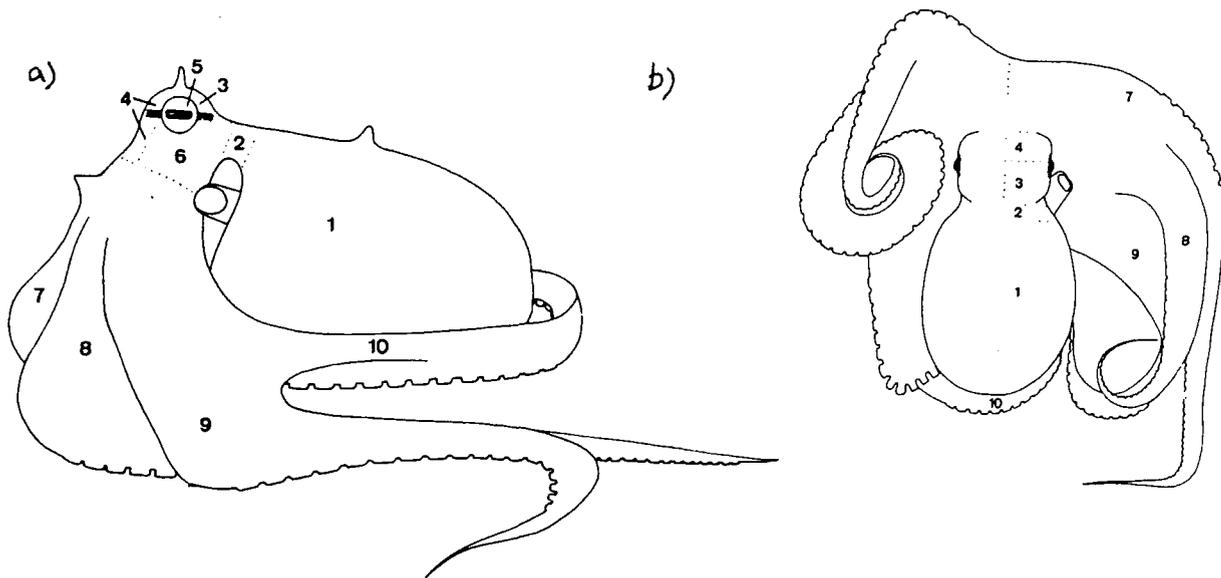


FIGURE 6. Pattern of projection of motor chromatophore fibres on the body surface of *O. vulgaris*: (a) lateral view; (b) dorsal view. Numbers 1-10 indicate the areas controlled by a separate brain nerve containing chromatophore fibres (Froesch, 1973).

#### 5.4. The nervous control system

The nerve cell bodies ( $\pm 150\,000$  in the adult) of the fibres innervating the chromatophores are housed in 4 discrete regions in the sub-oesophageal brain (Boycott 1953). These are the paired "anterior chromatophore lobes", whose nerve cells project to the head and arms, and the paired "posterior chromatophore lobes", whose nerve cells project to the mantle. There are inter-chromatophore connectives between the anterior and the posterior chromatophore lobes of each side. Each lobe innervates only the chromatophores on its own side. Probably, all of the chromatophore fibres run to the periphery without synapsing; the chromatophore lobes are therefore termed the "lower" motor centres (level 1) of the system (Andrews, Messenger & Tansey, 1983). These 4 lobes receive fibres from the statocysts, from the arms and mantle as well as a massive input from the small "lateral basal lobes".

The paired lateral basal lobes in the supra-oesophageal brain are "higher" motor centres (level 2) of the system. Each lateral basal lobe sends a tract (efferent) to the anterior and one to the posterior chromatophore lobe of its own side. They receive fibres from the median basal lobe, from the peduncle lobes and from the large "optic lobes". The paired optic lobes (level 3), each containing  $\pm 65\,000\,000$  neurones, act upon visual information gathered by the well-developed eyes. These lobes must be regarded as the ultimate controllers of chromatophore activity. The hierarchical nervous system is diagrammatically illustrated in figure 7.

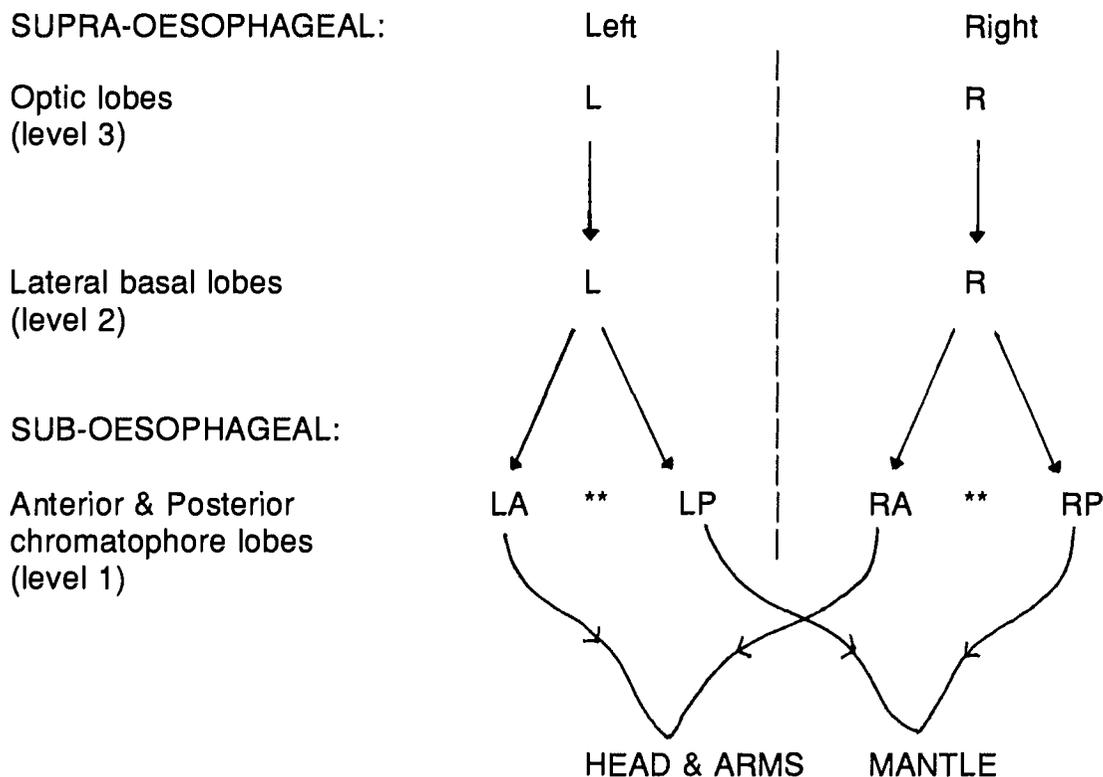


FIGURE 7. A diagram illustrating the hierarchy in the nervous system controlling the chromatophores of *Octopus* (\*\* represent inter-chromatophoral fibres).

The nervous system controlling the chromatophores in the skin can be excited by fibres coming from optic, tactile and static sense organs. Such afferent fibres probably work through the lateral basal lobes, although some tactile fibres may go direct to the sub-oesophageal chromatophore lobes (Boycott, 1953).

Boycott's (1961) electrical stimulation of the brain centres of the cuttlefish gave results which probably also hold for *Octopus*. Stimulation of a sub-oesophageal chromatophore lobe (level 1) at threshold intensity produces unilateral darkening; above threshold the darkening and papilla-raising spreads to the opposite side. Stimulation of the lateral basal lobes (level 2) produces blanching (which is thus a higher activity) as well as darkening and raising of papillae; unilateral responses are less frequent. Electrical stimulation of the optic lobes (level 3) produces full patterned responses, such as the sandy mottle colour, texture and warning spots.

Thus, the hierarchy in the nervous control of patterning becomes visible, from simple to complex, as one proceeds upwards in the brain.

## 5.5. Neurotransmitters

Andrews, Messenger & Tansey (1981; 1983) used a method by which small quantities of neurotransmitters were injected into the blood supplying the brain of cephalopods, to produce conspicuous and instantaneous colour changes in the skin of arms, head and body. The effects, particularly on the chromatophores, were studied.

Of the transmitter substances known to be present in the brain of cephalopods, dopamine, noradrenaline, octopamine, GABA and L-glutamate cause expansion of the chromatophores and darkening of the skin when injected. Acetylcholine (ACh) causes retraction of the chromatophores and paling; 5-hydroxy-tryptamine (5-HT) causes differential expansion and retraction, eliciting a mottled pattern. These responses, which are neurally mediated, are particularly well defined for the latter 2 substances. The three researchers used brain lesions to localise the sites of action of ACh and 5-HT; they suggest that these neurotransmitters act at the level of the sub-oesophageal lobes (level 1) to control the chromatophores, but 5-HT may also act at the level of the optic lobe (level 3) to control inking and defecation.

We have already seen that the control system for skin patterning in cephalopods is essentially hierarchical. This system is influenced by other regions of the brain, and is undoubtedly responsive to input from the arms and statocysts. However, it seems likely that it is the cell systems in the optic lobes, acting of course on visual information, that evoke the various patterns displayed by living octopods. Any skin patterning must result from selective excitation and/or inhibition exerted by centres higher in that hierarchy.

## 6. VISION IN CEPHALOPODS, PARTICULARLY IN *O.VULGARIS*

Octopods are excellent camouflagers and it is therefore highly surprising to find out that they are almost certainly colour-blind.

Since 1910, colour-vision in cephalopods has been studied in different genera, using different techniques, and several workers arrived at different conclusions. Before 1960, results of behavioural experiments were often inadequately quantified and serious errors were made in colour-vision experiments: researchers either failed to take into account the spectral sensitivity curve of the subject or failed to control for differences in brightness between test objects. Thus, early "evidence" on colour-vision in cephalopods is suspect. More recent investigations will be discussed in this section.

### 6.1. Electro-physiological experiments

Two Russian workers, Orlov and Byzov (1961; 1962) reported their findings on electro-physiological experiments of isolated retinae of two cephalopod species; *Ommastrephes sloanei-pacificus* (squid) and *O.dofleini*. They found that a given ERG (electro-retinogram) could be produced by light of different wavelengths if compensations for differences in brightness were made. The ERG was not affected when changing from one such illuminating wavelength to another. Thus, they did not find a so-called Purkinje-shift when switching between wavelengths, suggesting that the studied species are colour-blind.

A few years later, Hamasaki (1968a; 1968b) examined the ERG of intact, anaesthetised *Octopus briareus* and *O.vulgaris*. He found that the scotopic spectral sensitivity curve had a maximum at 480 nm, with no secondary peaks. Adaptation to white light, or to blue, green or red light, did not induce a shift in the maximum peak of the ERG. Again, the absence of a Purkinje-shift points to the presence of only one receptor system in the retinae of the octopods studied.

### 6.2. Photopigments and receptor cells

In several different cephalopods, only one photopigment has been characterised so far: rhodopsin. A second pigment has also been extracted, but this was not found to be a true photopigment (Messenger, 1979). The presence of one photopigment suggests the presence of one photosystem; this idea is confirmed by morphological studies on the cephalopod retina, which reveal only one class of receptors.

### 6.3. Behavioural (training) experiments

Octopods have proved themselves to be excellent subjects for learning experiments, which are used to gain insight in what the animals can see by training them to discriminate between different objects. The training technique commonly applied to *Octopus* is summarised as follows. The animal is first shown a figure cut out of perspex (the "positive" shape) and given a food reward for attacking it (healthy octopods readily swoop out and attack small, conspicuous, moving objects in the sea). Subsequently, a different ("negative") shape is presented but this time an attack leads to a small electric shock. Usually, the positive and the negative shape are introduced successively, in random order, for at least 20 trials per day. With simple discrimination, signs of learning become apparent on the second day of training; the animal will come swiftly and surely to the positive shape and slowly or not at all to the negative shape.

By the mid 1960's, experiments of this kind revealed that *O.vulgaris* can readily discriminate between objects differing in brightness, size, orientation and form. They also exhibit polarised light sensitivity and can discriminate between two lights, one with an e-vector horizontally and the other with an e-vector vertically (Messenger, 1979).

Messenger and his workers (Messenger, Wilson & Hedge, 1973; Messenger, 1979) designed several training experiments to study the vision of *O.vulgaris*. First, single cues were used, whereby octopods were trained to discriminate between two objects differing in hue, or two objects differing in relative brightness. The animals performed randomly on the hue discriminations but all learned to discriminate on the basis of brightness. Experiments using two cues, test-objects differing in hue and brightness, revealed that animals trained with brightness and hue trained very well but no better than animals trained with brightness alone. Thus, these different training experiments revealed that *O.vulgaris* quickly learns to discriminate on the basis of brightness, over the same period of time when it fails to discriminate between different hues. This strongly suggests that *O.vulgaris* is colour-blind.

### 6.4. Eye movements and optomotor responses

The results of the experiments on colour-vision discussed so far, strongly suggest colour-blindness for *Octopus*. However, there is still a possibility that the octopus eye is able to resolve differences in wavelength, but that this information is not made available for learning. To examine whether wavelength can be detected peripherally, at the level of the retina, more behavioural experiments were needed.

Octopods, like all animals with mobile eyes, will make compensatory movements if the external visual world moves. The classical experimental device for eliciting such responses is a drum, bearing black and white stripes, that can be rotated around the subject. If the drum turns clockwise, the eyes turn slowly clockwise but at a certain point they quickly flick back anti-clockwise, to begin slowly drifting clockwise again. This process, known as "nystagmus", is a method used by the animal to stabilise its retinal image. If this stabilisation cannot be achieved by eye movements alone, the head of the animal, and even its whole body, will turn in a similar manner when the drum rotates. Movements of the latter kind are termed "optomotor responses". A display of nystagmus or an optomotor response implies that the eye can detect discontinuity. If a perfectly uniform background is rotated around the subject, such responses need not be made. Indeed, *O. vulgaris* responds as predicted, with a revolving background of black and white stripes.

Colour-blindness was tested using the same apparatus, with stripes differing in hue but not in brightness. It was found that *O. vulgaris* gives clear and unequivocal nystagmus and optomotor responses to black and white stripes, to grey stripes differing only in brightness, and to "monochromatic" stripes differing only in brightness. No such responses were given to a plain grey background, nor to orange or blue stripes on a grey background (both the latter appeared uniformly grey to a human subject using rod vision). When the relative brightness of the stripes was altered (stripes thus differing in brightness) they were made discriminable to the octopus (Messenger, 1979).

The presence of nystagmus or optomotor responses does not necessarily mean that an animal has colour-vision, but the absence of such responses argues strongly for colour-blindness. It is therefore clear that if any information about wavelength is resolved peripherally, it by-passes, or fails to reach not only the visual memory store but also the centres for controlling the movement of the eyes, head, arms and body. It seems scarcely credible that the eye gathers such information but transmits this to the chromatophores alone, in order to form a matching dress with the background the animal is on.

Thus, taking together the results of electrophysiological investigations, the morphology of the retina and the behavioural experiments (discrimination training and nystagmus or optomotor responses), it is highly unlikely indeed that *O. vulgaris* perceives colours.

## 7. CAMOUFLAGE IN A COLOUR-BLIND ANIMAL

How do octopods manage to perfectly match their surroundings when they are colour-blind? This is the central question we shall be concerned with in this chapter.

### 7.1. Matching methods

The chromatophores are the most superficial of the three elements present in *Octopus* skin, which combine to produce cryptic colouration. Below them are the reflector cells, below these are the leucophores and underlying these is a layer of muscle. There are also muscle fibres running vertically in the skin; these enable the texture of the skin to be transformed from a smooth glassiness to an extreme roughness, sometimes with elevated papillae of 10 mm or more.

Octopods succeed in camouflaging themselves against a variety of natural backgrounds in the sea. On the output side this is achieved by neuro-muscular control of the skin and of the separate yellow, orange, red, brown and black chromatophores (Packard, 1978). The different colours to which the chromatophores in the skin of *Octopus* give rise are a combination of effects of the particular pigments present and the degree of expansion of the sac that contains them (i.e. of how thinly the pigment granules are spread).

Chromatophores can only give colour when illuminated, subtracting those wavelengths that do not contribute to the final hue. The light may either come from above the chromatophore or from below it, by reflection from the underlying structures, so that the colour is seen by transparency. The hue that is perceived will depend on the proportion in which the visible wavelengths are present in the light that illuminates the pigment body (similarly, chromatophores of a particular hue illuminated by light deficient in that hue will appear black, as do red chromatophores when seen at depths below 20-30 m).

Any structures, such as reflector cells, lying below the chromatophore will add their own colours and if these are complementary to those characteristic of the chromatophore pigment they will result in colours of intermediate hue. The brightness of the colours seen by transparency will depend on how much light is being reflected through the chromatophore. This is partly a matter of the efficiency of light-scattering and reflecting structures below them - leucophores being more efficient than muscle in this respect - and partly a matter of how much light is available for reflection.

A screen of melanophores will absorb most of the incident light of all wavelengths and thus leave little light to be reflected by structures beneath. Such a screen will act as a "neutral density filter", enhancing or reducing reflectance from the structures lying underneath. The neutral density role of melanophores is emphasised by their usual placing in the skin as the most superficial layer of elements involved in skin patterning. Finally, the appearance of chromatophores will depend on their orientation relative to the light, whether incident or reflected (Packard & Hochberg, 1977).

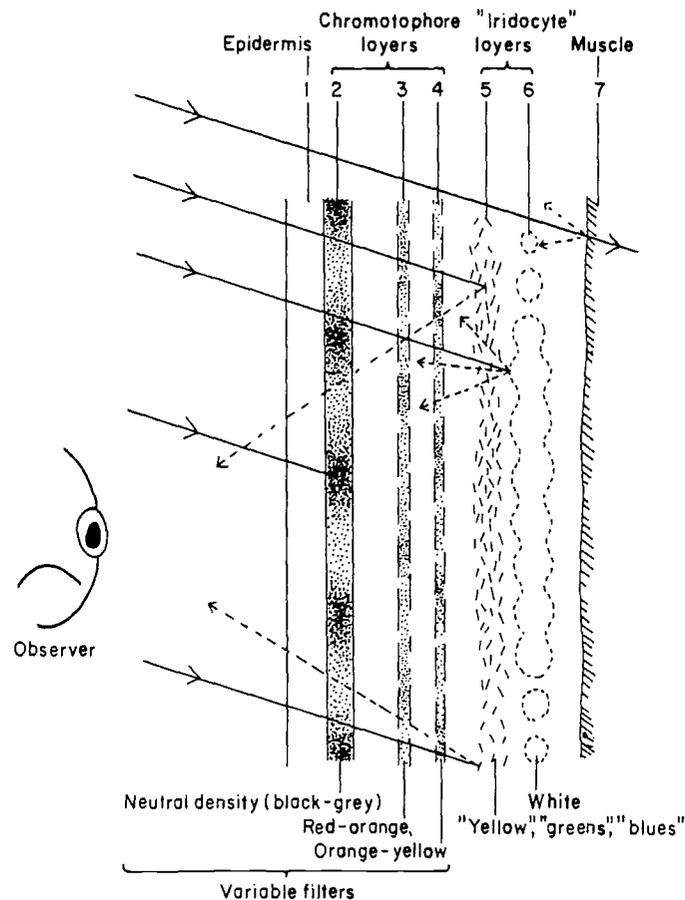


FIGURE 8. Schematic view of *Octopus* skin to illustrate its optical properties with respect to an observer. Although generalized, the diagram may be regarded as covering an area of a single patch and its bounding grooves cut normal to the surface. Incident light passes successively through a transparent refracting layer 1 and neutral density and colour filters 2, 3 and 4 before being reflected or absorbed by layers 5, 6 and 7. Rays of light are shown as if coming from one direction only; refraction is not indicated. The variable filters are under nervous control. The neutral density filter 2 is formed of melanophores and may be tinted brown or red at the dark end of its range. Peak absorption in the colour filters 3 and 4 shifts towards the short end of the spectrum as they expand. The mirror-reflecting layer 5 reflects light of narrow band width in directions that depend on the orientation of the reflecting platelets composing it; only a few orientations are shown. The strongly reflecting white backing layer 6 scatters as well as reflects; it is composed of leucophores (Packard & Hochberg, 1977).

We can recognise 2 extreme conditions:

- 1) Melanophores, expanded or piled up (as on the side walls of a groove), are embedded in transparent skin not underlain by any reflecting structures. This gives black.
- 2) No chromatophores present, or those present are fully retracted and the transparent skin contains totally reflecting and diffusing structures, principally leucophores and body musculature. This gives white.

It is possible to demonstrate that there can be a better match for wavelength on the side of an octopus where there is no chromatophore activity (obtained by sectioning the pallial nerve) than on the other side. It is also easy to show that an octopus in a dish of coloured stones or on plain coloured paper does not turn a uniform colour all over, nor does it really "change colour" when placed on a differently coloured background: it pales or darkens, only incidentally acquiring a tinge of the background colour (Messenger, 1974).

Between the 2 extremes - extremes of intensity, not hue - all variations (both of intensity and hue) are possible. It is the job of the reflector cells to give hues of blue and green, and of the pigment bodies of the chromatophores to give hues of yellow, orange, reds and browns of different intensity (i.e. tone).

There seem to be at least three methods used by *Octopus* to match its immediate surroundings (Messenger, Wilson & Hedge, 1973):

- 1) The transmission of reflected light.  
This applies only to very young animals whose skin, particularly along the arms, is very thin and transparent. In simple experiments with coloured stones it is easy to demonstrate that the predominant wavelength reflected off the background will be transmitted through the skin, especially if all the chromatophores are contracted.
- 2) The reflection of incident light.  
The skin of *Octopus* contains chromatophores, reflector cells and leucophores. The latter two elements are good reflectors of incident light, whether it be white or a "monochromatic" colour. The reflector cells also give "structural" colours by constructive interference. Thus, an octopus has only to contract its chromatophores to reveal a reflecting system that can automatically match the background.

- 3) The reflection of incident light after absorption.  
In *Octopus*, there are differently coloured (yellow, orange, red, brown and black) chromatophores. When these are expanded, and white light falls upon them, they strongly absorb the shorter wavelengths. The chromatophores can be expanded differentially, so that black, brown, red, orange or yellow dots can be produced. The yellow-red region of the spectrum matches brownish, rocky backgrounds. Furthermore, this system of dots permits excellent tone-matching, contributing to the success of cryptic "colouration".

Thus, *Octopus* can match its background by a combination of devices: transmission, passive reflection and reflection with differential absorption. The first two processes operate automatically when the chromatophores are "switched-off" (retracted) and give a good colour-match. The third may act principally by producing a good intensity-match; this can be achieved with information from a colour-blind eye.

Therefore, we see that for *Octopus* to be able to match the background reflectance requires only that its eye is sensitive to brightness, and we have seen that there is good evidence that this is so. Presumably the background reflectance (or albedo) is estimated and the tonus of the chromatophore muscles adjusted to give the skin the appropriate luminance. Experiments are needed to test the theory that the programme controlling the chromatophores is regulated by the eye on the basis of albedo, but we may note the following: octopods are pale in bright light and turn dark as the light decreases, and blinded octopods are invariably far darker than their background (Messenger, 1979).

In dim light the eye registers a low albedo and cells in the optic lobes will initiate a programme that will tonically expand the chromatophores, mostly the superficial melanophores, so as to diminish the luminance. Little light will now reach the reflector cells and leucophores and what little is reflected from them will be filtered by the overlying screen of chromatophores.

In bright light, however, the high reflectance will elicit a different programme, reducing the tone in the chromatophore muscles so that the chromatophores will partially retract. This will expose the underlying reflecting system of reflector cells and leucophores, which then reflect the colour of the incident light. This camouflage system led Messenger to formulate the following thesis: "If *Octopus* takes care of the luminance, the colour takes care of itself".

## 7.2. Countershading reflex

An interesting temporary camouflage reflex has recently been described by Ferguson & Messenger (1991). Most cephalopods are seen to have more chromatophores on the dorsal body surface than on the ventral, and these dorsal chromatophores tend to be kept tonically expanded. As a result, the dorsal surface is usually darker than the ventral, an effect known as countershading.

When *Sepia officinalis*, *Loligo vulgaris* and *O. vulgaris* are rotated 180° around their longitudinal body axis, the ventral chromatophores expand (causing darkening) while the dorsal chromatophores retract (causing paling). When the animals are rotated through only 90°, the chromatophores on the uppermost half of the ventral and dorsal surface expand, whilst those on the lower half retract. This response, which Ferguson & Messenger term the "countershading reflex", can be abolished by removal of the statocysts: experiments in which the direction of incident light is reversed, show that this reflex is not driven by sensory input from the eye. The function of the reflex is, presumably, to maintain countershading whilst the animal is momentarily disoriented (e.g. by a predator); this idea is supported by the fact that the reflex lasts only a few seconds.

The countershading reflex is accompanied by another reflex response to disorientation; funnel pointing. The funnel always points upwards, towards the water surface, when the animal is rotated. Funnel pointing is directly driven by receptors in the statocysts. It is probably useful for rolling the animal back towards an even keel when it is disorientated, by the expulsion of a jet of water in an upward direction.

## 8. CONCLUSIVE REMARKS

After having introduced camouflage in cephalopods, we have taken a closer look at *O. vulgaris*, the real "chameleon of the sea". Skin patterning in this species was studied at different levels: whole patterns, light and dark components making up patterns, patch and groove units making up components, and different elements present in the dermis. Patterning of the skin is neurally controlled, so that details on the nervous system could not be excluded. Remarkably, this fantastic camouflager was found to be colour-blind, but even with such a sensory deficiency it is able to utilise the different structures in its skin to obtain a perfect match with the immediate surroundings.

The question that still remains is "why does *Octopus* need such elaborate devices for concealment?"

Perhaps the main reason is that, although it is an active animal, it is also relatively slow-moving and clumsy. It has lost the external, protective shell that its ancestors were equipped with and must therefore defend itself by other, pictorial means (Packard & Sanders, 1969). Octopods had to protect themselves from a group of animals with excellent colour vision: the bony fish. Any brightness matching system that early, soft-skinned cephalopods acquired might have been adequate to protect themselves from other colour-blind cephalopods, but a poor colour match may not have deceived a fish. Thus, those octopods that acquired a way of mimicking colour (e.g. by developing reflectors) would have been at an advantage. Protective resemblance, once developed, also allows *Octopus* to hide from its prey and so to adopt surprise tactics (Messenger, 1979).

*Octopus*, like many other cephalopods, does not only use its skin for camouflage purposes. Any skin pattern which is clearly visible is potentially available for signalling, and many cephalopods have indeed developed visual signalling. Of course intra-specific displays are framed in terms of differences in brightness, rather than being colour displays.



## LITERATURE

- Andrews, P.L.R.; Messenger, J.B. & Tansey, E.M. (1981) -**  
Colour changes in cephalopods after neurotransmitter injection into the cephalic aorta.  
*Proc.R.Soc.Lond. B.* **213**: 93-99.
- Andrews, P.L.R.; Messenger, J.B. & Tansey, E.M. (1983) -**  
The chromatic and motor effects of neurotransmitter injection in intact and brain-lesioned *Octopus*.  
*J.Mar.Biol.Ass. U.K.* **63**: 355-370.
- Andrews, P.L.R.; Packard, A. & Tansey, E.M. (1982) -**  
A physiologically discrete population of chromatophores in *Octopus vulgaris* (Mollusca).  
*J.Zool.Lond.* **198**: 131-140.
- Barnes, R.D. (1987) -**  
Invertebrate Zoology - Fifth Edition. Saunders College Publishing.  
*Editors: Holt, Rinehart & Winston.* Dryden Press (Philadelphia).
- Barth, R.H. & Broshears, R.E. (1982) -**  
The Invertebrate World - First Edition. Saunders College Publishing.  
*Editors: Holt, Rinehart & Winston.* Dryden Press (Philadelphia).
- Boycott, B.B. (1953) -**  
The chromatophore system of cephalopods.  
*Proc.Lin.Soc.Lond.* **164**: 235-240.
- Boycott, B.B. (1961) -**  
The functional organization of the brain of the cuttlefish *Sepia officinalis*.  
*Proc.R.Soc.Lond. B.* **153**: 503-534. Taken from: Packard & Sanders (1969).
- Boyle, P.R. & Dubas, F. (1981) -**  
Components of body pattern displays in the octopus *Eledone cirrhosa* (Mollusca: Cephalopoda).  
*Mar.Behav.Physiol.* **8**: 135-148.
- Bozler, E. (1931) -**  
Über der tätigkeit der einzelnen glatten muskelfaser bei kontraktion. III. Mitt. Registrierung der kontraktionen der chromatophoren-muskelzellen von cephalopoden. *Z.verg.Physiol.* **13**: 762-772.  
Taken from: Cloney & Florey (1968).
- Brocco, S.L. (1975) -**  
The fine structure of the frontal and mantle white spots of *Octopus dofleini*.  
*American Zoologist* **15(3)**: 782.
- Brocco, S.L. (1977) -**  
The ultrastructure of the epidermis, dermis, iridophores, leucophores and chromatophores of *Octopus dofleini martini* (Cephalopoda: Octopoda). *Ph.D. Thesis, University of Washington, Seattle*. Taken from: Brocco & Cloney (1980).
- Brocco, S.L. (1981) -**  
The ultrastructure of the dermal leucophores of *Octopus dofleini*.  
*Cephalopod Newsletter* **5**: 14.
- Brocco, S.L. & Cloney, R.A. (1980) -**  
Reflector cells in the skin of *Octopus dofleini*.  
*Cell Tissue Res.* **205**: 167-186.
- Cloney, R.A. & Florey, E. (1968) -**  
Ultrastructure of cephalopod organs.  
*Z.Zellforsch.* **89**: 250-280.

- Denton, E.J. & Land, M.F. (1971) -**  
Mechanism of reflexion in silvery layers of fish and cephalopods.  
*Proc.R.Soc.Lond. A.* **178**: 43-61.
- Dubas, F. (1987) -**  
Innervation of chromatophore muscle fibers in the octopus *Eledone cirrhosa*.  
*Cell Tissue Res.* **248**: 675-682.
- Dubas, F. & Boyle, P.R. (1985) -**  
Chromatophore motor units in *Eledone cirrhosa* (Cephalopoda: Octopoda).  
*J.Exp.Biol.* **117**: 415-431.
- Ferguson, G. & Messenger, J.B. (1991) -**  
A countershading reflex in cephalopods.  
*Proc.R.Soc.Lond. B.* **243**: 63-67.
- Fioroni, P. (1965) -**  
Die embryonale musterentwicklung bei einigen mediterranen tintenfischarten.  
*Vie et Milieu* **16**: 655-756. Taken from: Packard (1985).
- Fioroni, P. (1970) -**  
Die embryonale genese der chromatophoren bei *Octopus vulgaris*.  
*Acta anatomica* **75**: 199-224.
- Florey, E. (1966) -**  
Nervous control and spontaneous activity of the chromatophores of a cephalopod, *Loligo opalescens*. *Comp.Biochem.Physiol.* **18**: 305-324. Taken from: Cloney & Florey (1968).
- Florey, E. (1969) -**  
Ultrastructure and function of cephalopod chromatophores.  
*American Zoologist* **9**: 429-442.
- Froesch, D. (1973) -**  
Projection of chromatophore nerves on the body surface of *Octopus vulgaris*.  
*Marine Biology* **19**: 153-155.
- Froesch, D. & Messenger, J,B, (1978) -**  
On leucophores and the chromatic unit of *Octopus vulgaris*.  
*Zool.Lond.* **186**: 163-173.
- Froesch, D & Packard, A. (1979) -**  
*Octopus* chromatophores accumulate nickel.  
*Experientia (Basel)* **35(6)**: 828-830.
- Froesch-Gaetzi, V. & Froesch, D. (1977) -**  
Evidence that chromatophores of cephalopods are linked by their muscles.  
*Experientia (Basel)* **33(11)**: 1448-1450.
- Grzimek, B. (1973) -**  
Het leven der dieren - Encyclopedie van het dierenrijk. Deel III - Weekdieren en Stekelhuidigen. *Uitgeverij Het Spectrum* (Utrecht/Antwerpen) MCMLXXIII.
- Hamasaki, D.I. (1968a) -**  
The electro-retinogram of the intact anaesthetized *Octopus*.  
*Vision Research* **8**: 247-258.
- Hamasaki, D.I. (1968b) -**  
The ERG-determined spectral sensitivity of the *Octopus*.  
*Vision Research* **8**: 1013-1021.

- Hanlon, R.T. (1982) -**  
The functional organization of chromatophores and iridescent cells in the body patterning of *Loligo plei* (Cephalopoda: Myopsida). *Malacologia* **23(1)**: 89-119.
- Mangold, K. (1983) -**  
Cephalopod Life Cycles, Volume 1: Species Accounts. Chapter 21. *Octopus vulgaris*.  
*Edited by Boyle, P.R.* Academic Press (London).
- Meinhardt, H. & Gierer, A. (1974) -**  
Applications of a theory of biological pattern formation based on lateral inhibition.  
*J.Cell.Sci.* **15**: 321-346. Taken from: Packard (1985).
- Messenger, J.B. (1974) -**  
Reflecting elements in cephalopod skin and their importance for camouflage.  
*J.Zool.Lond.* **174**: 387-395.
- Messenger, J.B. (1979) -**  
The eyes and skin of *Octopus*: compensating for sensory deficiencies.  
*Endeavour (Oxford)* **3(3)**: 92-98.
- Messenger, J.B.; Wilson, A.P. & Hedge, A. (1973) -**  
Some evidence for colour-blindness in *Octopus*.  
*J.Exp.Biol.* **59**: 77-94.
- Mirow, S. (1972a) -**  
Skin color in the squids *Loligo pealii* and *Loligo opalescens*: I. Chromatophores.  
*Z.Zellforsch.* **125**: 143-175.
- Mirow, S. (1972b) -**  
Skin color in the squids *Loligo pealii* and *Loligo opalescens*: II. Iridophores.  
*Z.Zellforsch.* **125**: 176-190.
- Orlov, O.Yu. & Byzov, A.L. (1961) - (In Russian) -**  
Colorimetric research on the vision of molluscs (Cephalopoda).  
*Dokl.Akad.Nauk. SSSR* **139**: 723-725. Taken from: Messenger, Wilson & Hedge (1973).
- Orlov, O.Yu. & Byzov, A.L. (1962) - (In Russian) -**  
Vision in cephalopod molluscs.  
*Priroda, Mosk.* **3**: 115-118. Taken from: Messenger, Wilson & Hedge (1973).
- Packard, A. (1978) -**  
Cracking the code of the camouflage patterns of the common octopus, *Octopus vulgaris*.  
*Neuroscience Letters Suppl.* **(1)**: S 133.
- Packard, A. (1982) -**  
Morphogenesis of chromatophore patterns in cephalopods: are morphological and physiological 'units' the same? *Malacologia* **23(1)**: 193-201.
- Packard, A. (1985) -**  
Sizes and distribution of chromatophores during post-embryonic development in cephalopods.  
*Vie Milieu* **35(3/4)**: 285-298.
- Packard, A. & Hochberg, F.G. (1977) -**  
Skin patterning in *Octopus* and other genera.  
*Symp.Zool.Soc.Lond.* **38**: 191-231.
- Packard, A. & Sanders, G. (1969) -**  
What the *Octopus* shows to the world.  
*Endeavour* **28**: 92-99.
- Packard, A. & Sanders, G.D. (1971) -**  
Body patterns of *Octopus vulgaris* and maturation of the response to disturbance.  
*Animal Behaviour* **19**: 780-790.

**Sanders, G.D. & Young, J.Z. (1974) -**

Reappearance of specific colour patterns after nerve regeneration in *Octopus*.  
*Proc.R.Soc.Lond. B.* **186**: 1-11.

**Sangiovanni, G. (1891) -**

Title unknown. *Giornale enciclopedico di Napoli* **13**: N.9.  
Taken from: Sereni (1930).

**Sereni, E. (1930) -**

The chromatophores of the cephalopods.  
*Biol.Bull.* **59**: 247-268.

**Wells, M.J. (1978) -**

Octopus - Physiology and behaviour of an advanced invertebrate. First edition.  
*Editors: Chapman and Hall* (London).

**Wicksten, M.K. (1983) -**

Camouflage in marine invertebrates.  
*Oceanogr.Mar.Biol.Ann.Rev.* **21**: 177-193.

