

Cephalopod chromatophores: neurobiology and natural history

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ABSTRACT

The chromatophores of cephalopods differ fundamentally from those of other animals: they are neuromuscular organs rather than cells and are not controlled hormonally. They constitute a unique motor system that operates upon the environment without applying any force to it. Each chromatophore organ comprises an elastic sacculus containing pigment, to which is attached a set of obliquely striated radial muscles, each with its nerves and glia. When excited the muscles contract, expanding the chromatophore; when they relax, energy stored in the elastic sacculus retracts it. The physiology and pharmacology of the chromatophore nerves and muscles of loliginid squids are discussed in detail. Attention is drawn to the multiple innervation of dorsal mantle chromatophores, of crucial importance in pattern generation. The size and density of the chromatophores varies according to habit and lifestyle. Differently coloured chromatophores are distributed precisely with respect to each other, and to reflecting structures beneath them. Some of the rules for establishing this exact arrangement have been elucidated by ontogenetic studies. The chromatophores are not innervated uniformly: specific nerve fibres innervate groups of chromatophores within the fixed, morphological array, producing ‘physiological units’ expressed as visible ‘chromatomotor fields’.

The chromatophores are controlled by a set of lobes in the brain organized hierarchically. At the highest level, the optic lobes, acting largely on visual information, select specific motor programmes (i.e. body patterns); at the lowest level, motoneurons in the chromatophore lobes execute the programmes, their activity or inactivity producing the patterning seen in the skin. In *Octopus vulgaris* there are over half a million neurons in the chromatophore lobes, and receptors for all the classical neurotransmitters are present, different transmitters being used to activate (or inhibit) the different colour classes of chromatophore motoneurons. A detailed understanding of the way in which the brain controls body patterning still eludes us: the entire system apparently operates without feedback, visual or proprioceptive.

The gross appearance of a cephalopod is termed its body pattern. This comprises a number of components, made up of several units, which in turn contains many elements: the chromatophores themselves and also reflecting cells and skin muscles. Neural control of the chromatophores enables a cephalopod to change its appearance almost instantaneously, a key feature in some escape behaviours and during agonistic signalling. Equally important, it also enables them to generate the discrete patterns so essential for camouflage or for signalling. The primary function of the chromatophores is camouflage. They are used to match the brightness of the background and to produce components that help the animal achieve general resemblance to the substrate or break up the body’s outline. Because the chromatophores are neurally controlled an individual can, at any moment, select and exhibit one particular body pattern out of many. Such rapid neural polymorphism (‘polyphenism’) may hinder search-image formation by predators. Another function of the chromatophores is communication. Intraspecific signalling is well documented in several inshore species, and interspecific signalling, using ancient, highly conserved patterns, is also widespread. Neurally controlled chromatophores lend themselves supremely well to communication, allowing rapid, finely graded and bilateral signalling.

Key words: cephalopods, chromatophores, pigments, body patterning, motor system, camouflage, signalling, vision.

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I. INTRODUCTION

It has been known since antiquity that cephalopods can change their appearance, or, following current convention, their *body pattern*, swiftly and dramatically. The organs principally responsible for this – the chromatophores – were first accurately described 180 years ago; they have subsequently been studied by biologists of all kinds and in the last 30 years their organization has been analysed at all levels from the subcellular to the social. The ability of cuttlefish, squids and octopuses to change colour instantly must have been known to Mediterranean fishermen from time immemorial: certainly Aristotle (translation 1910) wrote about it in *Historia Animalium*.

However it was not until the nineteenth century that the mechanism of such colour change began to be properly investigated. The earliest serious investigations were carried out by two Neapolitans, Giosuè Sangiovanni (1819, 1829) and Stefano Delle Chiaje (1829). Sangiovanni's brilliantly perceptive earlier paper shows an amazing understanding of how chromatophores work: above all he recognised that the cephalopod skin contains many thousands of tiny small organs, the 'organi cromoforo espansivo-dermoideo'. Delle Chiaje's great contribution was to show that it was the radial muscles that expanded the 'chromophore' (see below).

These important discoveries were either overlooked or not accepted by the innumerable biologists from all over Europe who studied chromatophores during the late nineteenth century, again at Naples, where the Zoological Station had been founded in 1873. Some idea of the extent of the early literature on cephalopod chromatophores can be gained from the fact that van Rynberk's (1906) review cites over 80 references. Much of this work is difficult to follow because of its length, the discursive narrative style of the authors of that period, usually writing in German, and the total absence of figures! Moreover the polemical stance taken by the authors often makes interpretation difficult. Florey (1969) has pointed out that at the turn of the century there was a fundamental split between those physiologists who thought that the chromatophore was passive and the

radial muscle fibres active, and those who regarded the fibres merely as elastic connective tissue strands, attributing to the chromatophore itself the power of expansion and retraction. There was also debate about whether the entire chromatophore with all its radial muscles was a syncytium, for chromatophores are often seen to pulsate, even in the absence of nervous stimulation (see Section II.5). The careful observations of workers such as Hofmann (1907*a-c*; 1910*a, b*) and Bozler (1928, 1929, 1930), working without the benefits of electron microscopy or modern electrophysiological equipment, seemed irreconcilable. It was not until the late 1960s that Florey and his co-workers resolved these contradictions, and firmly established the basis of our present-day understanding of the functional organisation of the squid chromatophore organ, which is considered in Section II.

The organization of populations of chromatophores began to be investigated in the 1970s, when morphological studies revealed the complexity of the skin's organization for the first time (Mirow, 1972*a, b*; Froesch & Messenger, 1978) and electrical stimulation demonstrated the non-random nature of the chromatophore motor fields in, first, *Octopus vulgaris* (Packard, 1974) and subsequently in the squid *Lolliguncula brevis* (Ferguson, Martini & Pinsker, 1988) (Section III).

The development of the chromatophores, first studied by Naef (1921, 1928), has been studied during embryogenesis by Fioroni (1965) and his colleagues (Poggel & Fioroni, 1986), and at the level of the young, whole animal by Packard (1982, 1985); the development of patterning over a lifetime has been followed in the cuttlefish, *Sepia officinalis*, by Hanlon & Messenger (1988). These topics are dealt with in Section IV.

Meanwhile, Sereni (1930) had shown how certain pharmacological agents perfused into the circulatory system of octopuses could induce colour changes; Sereni & Young (1932) had established that the chromatophores were innervated directly from the brain; Holmes (1940) had shown the complexity of body patterning in the cuttlefish; and Boycott (1953, 1961) had identified the chromatophore system in its brain. The nature of the central control of the

chromatophores was later explored by, among others, Chichery & Chanelet (1976, 1978), Dubas *et al.* (1986*a*), and Andrews, Messenger & Tansey (1983). This work is considered in Section V.

At about the same time as Florey was examining the physiology of squid chromatophores, Packard was beginning his important studies of chromatophores at the level of the whole animal, showing how the body pattern were built up hierarchically from components that, in turn, comprise units made up of different elements (Packard & Sanders, 1969, 1971: Section VI). Packard (1972) was also quick to draw attention to the way in which the chromatophores were adapted to the vertebrate visual system that 'designed' them (Section VIII). Meanwhile the discovery that octopuses were almost certainly colour-blind (Messenger, Wilson & Hedge, 1973) had prompted re-examination of the camouflage techniques that cephalopods employ for concealment (Section VII); and the ethological studies of Moynihan (1975; Moynihan & Rodaniche, 1977, 1982) had drawn attention to the importance of the chromatophores for signalling in such social cephalopods as the reef squid, *Sepioteuthis sepioidea* (Section VII), work later extended by Hanlon and his colleagues (e.g. Di Marco & Hanlon, 1997).

This review attempts to bring together these different lines of investigation. As so often in biology this will involve analysis at many different levels: from the subcellular, to the whole organ, to the whole animal and finally to the social level, as we consider how these animals communicate with each other using the chromatophores.

Before beginning, however, it needs emphasizing how different the chromatophores of a cephalopod are from those of a crustacean, fish, amphibian or reptile. In all these animals, the term chromatophore refers to a branched cell within which pigment granules can move: the control of such movements is commonly endocrine, although in some groups there is neural control and in others there are both kinds. In cephalopods, however, the chromatophores are organs and they function without any endocrine influence whatsoever.

II THE CHROMATOPHORE ORGANS

(1) Morphology

Figure 1A is the well-known, much quoted, diagrammatic representation of the chromatophore organ of the California market squid, *Loligo opalescens*

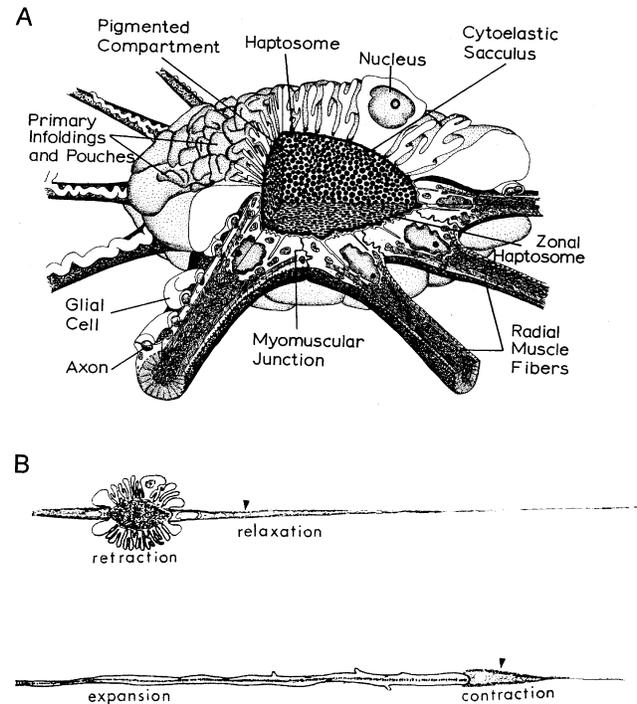


Fig. 1. (A) The first diagram of a chromatophore organ based on electron microscopy: Florey's 'classic' picture of a retracted chromatophore from the squid, *Loligo opalescens*. For simplicity the external lamina and the sheath are omitted and only a few radial muscle fibres are shown (Cloney & Florey, 1968). (B) Vertical section through a retracted and expanded chromatophore, showing one of its radial muscles (arrow), respectively relaxed and contracted (Florey, 1969).

(Cloney & Florey, 1968). It comes from the first ultrastructural investigation of a cephalopod chromatophore and its essential accuracy has since been confirmed by a number of other workers, notably Mirow (1972*a*), Froesch (1973*a*), and Reed (1995*b*). Cloney & Florey (1968) established that there are five different cell types in the chromatophore organ and it is convenient to deal with each of these in turn.

(a) *The chromatophore proper and its pigments*

At the centre of each chromatophore there is a single cell containing a nucleus peripherally, smooth endoplasmic reticulum, a few mitochondria and, most conspicuously, a large, pigment-bearing compartment containing many pigment granules. In *Loligo opalescens* these are discrete, membrane-bound inclusions, which are ellipsoidal (in yellow or red chromatophores) or roughly spherical (in brown chromatophores) (Cloney & Florey, 1968). The pigment imparts colour to the chromatophore

organ: yellow, orange, red, brown (or black). There are apparently no pigmentary blues or greens in cephalopods. It needs emphasising that there are differences between the chromatophores of different species (Fig. 2, Plate 1). For example, *Loligo opalescens* has yellow, red and brown chromatophores (Cloney & Florey, 1968), as has *Sepia officinalis* (Hanlon & Messenger, 1988); but another loliginid, *Alloteuthis subulata*, has only yellow and red chromatophores (Cornwell, Messenger & Hanlon, 1997), while *Octopus vulgaris* has yellow, orange, red, brown and also black chromatophores (Packard & Hochberg, 1977).

Chemically the pigments are most commonly ommochromes (Schwinck, 1956); these produce yellow, orange, red and brown pigments that may all belong to a single biochemical series derived from the oxidation of tryptophan (Fox & Vevers, 1960). Van Den Branden & Declair (1976) have partly characterized three pigments extracted from the dorsal skin of *Sepia officinalis*. In *Octopus vulgaris* there are also black chromatophores (Fig. 16, Plate 2). Here the pigment may be melanin (eumelanin): the study of Fox & Crane (1942) claimed there were considerably higher levels of melanin in the skin of *O. bimaculatus* than in the squid *Loligo opalescens*. This has never been confirmed, however, and Packard & Hochberg (1977), who describe how some chromatophores arise as clear spheres but within a few days 'progressively darken and pass through orange to deep red and eventually muddy brown on their way to black', implicitly question the presence of melanin. Froesch & Packard (1979) have also shown, in *Octopus vulgaris*, that the youngest, pale yellow chromatophores lie deepest in the skin whereas the oldest, black chromatophores occur most superficially and that the levels of zinc in the pigment granules increase with chromatophore age and darkening. The whole question of the nature of the chromatophore pigments merits re-examination.

Incidentally, authors have sometimes employed the term 'melanophore' (e.g. Packard & Hochberg, 1977), but since this has been used to refer to dark red as well as to black chromatophores, and since it has never gained wide acceptance in the cephalopod literature the term seems best avoided with these animals.

The bag containing the pigment granules, which is smooth and composed of fine filamentous material, was, for reasons made clear below, termed the *cytoelastic sacculus* by Cloney & Florey (1968). It is surrounded by the chromatophore cell membrane, which is attached to the sacculus surface by a series

of focal haptosomes (Gr. *haptein*, to fasten). This is extraordinarily folded in the retracted state, but is unfolded and stretched thinly over the pigment sac when the radial muscles contract and expand the chromatophore (Fig. 1B). The two layers of the sacculus, an outer, superficial layer and an inner, deeper layer, insert on the cytoplasmic surface of the plasmalemma opposite the zone of attachment to the radial muscle fibres, which are termed zonal haptosomes. Horizontal sections of the sacculus reveal that it contains large and small microfilaments; the larger (diameter 24 nm) are clearly oriented in all axes and the finer filaments form a dense felt 'mat'.

There is compelling evidence that the cytoelastic sac does not actively contract to cause chromatophore retraction; instead its elastic properties lead to chromatophore retraction after the radial muscles have relaxed (*ibid*; Cloney & Brocco, 1983). Thus there are very few mitochondria in the chromatophore proper (in contrast to the mitochondria-rich radial muscles); the sacculus never folds; the sacculus is strategically attached to the radial muscles; and exposure of cephalopod skin to ammonia fumes leads to complete retraction of all the chromatophores (Hofmann, 1907*c*). Moreover Cloney & Brocco (1983) found no evidence of glycogen in the chromatophore; and Froesch (1974) showed that cytochalasin B, known to interfere with microfilament arrays, did not abolish the elastic properties of the sacculus when applied to *Octopus vulgaris* skin.

(b) Radial muscles

There is a set of between 15 and 25 flat, wedge-shaped radial muscles lying around the chromatophore proper and attached to its margins at the myochromatophoral junctions. Distally each muscle comprises a central core of mitochondria surrounded by myofilaments: this forks into two proximally, close to the pigment sac (Reed, 1995*b*). The muscles are obliquely striated (Cloney & Florey, 1968; Weber, 1968) and show conventional twitch and tetanus responses (see below): they are unusual, however, in that they are electrically coupled to their neighbours. Cloney & Florey (1968) described very narrow (3 nm) myomuscular junctions between adjacent muscle fibres (Fig. 1); and Florey & Kriebel (1969) were able to measure the low specific resistance of the junctions. Recently, Reed (1995*a*) has confirmed that the muscles are coupled by iontophoretically injecting Lucifer Yellow into a single fibre (Fig. 3, Plate 1); moreover, dye transfer can be blocked by octanol. In her preparations, she

found that less than half the muscle fibres were dye-coupled; furthermore in over a third of coupled muscles dye passed to one side only of the impaled muscle. The significance of these findings has still not been fully elucidated: coupling must facilitate expansion of the whole chromatophore after activation of only a few radial muscles, which may be useful when gross, fast chromatophore expansion is called for. In the fully uncoupled situation, by contrast, exquisitely fine control of expansion is possible as separate muscle fibres contract independently (Florey & Kriebel, 1969). Clearly the possibility exists that there may be a mechanism for coupling or uncoupling the radial muscles as required, and Reed (1995a) has some preliminary evidence that high levels of Ca^{2+} close the gap junctions, leading to uncoupling.

(c) Chromatophore nerves

In loliginid squids, the association of nerves with the muscles has been known for over 90 years (at the level of the light microscope) and it is interesting to compare the Methylene Blue staining of Hofmann (1907b) with that of an antibody to L-glutamate (Messenger, Cornwell & Reed, 1997) (Fig. 4, Plate 1). It should be noted, however, that in octopods the arrangement is quite different: nerves tend to run across the muscle fibre forming *en passant* synapses, and the physiology of octopod chromatophores may be very different from that described below for squids. Unfortunately their small size precludes intracellular investigation (Froesch, 1973a; Dubas, 1987).

With the electron microscope, Cloney & Florey (1968) showed that each radial muscle was innervated by at least one nerve branch, generally more (see also Weber, 1968, 1973), and that these followed a serpentine course along the radial muscle, possibly to accommodate the great changes in length of the muscle as it contracts. They also described vesicles in the axon, 50–70 nm in diameter, but give no details of the synapses. Mirrow (1972a) found two types of vesicle, electron-lucent and electron-dense, ranging in size from 30 to 50 nm diameter, although it is not clear whether these were in the same axon. She also made the interesting point that the muscle was innervated along its length rather than at specialized sites.

More recently the important study of Reed (1995b) on *Loligo vulgaris* has added considerably to our understanding of the innervation of the radial muscles of squids. On the basis of serial ultra-thin

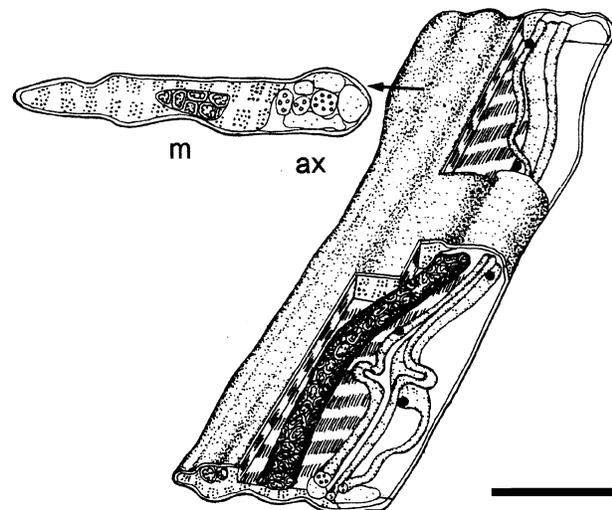


Fig. 5. Reconstruction of part of a relaxed yellow chromatophore muscle fibre of *Loligo vulgaris*, based on serial ultrathin sections. The enlargement (next to the arrow) shows a profile of the muscle at the point indicated, with the central core of mitochondria (m) and the three axons (ax) to the right. Dots (●) represent synapses. Scale bar, 10 μm (modified from Reed, 1995b).

sections along the entire length of the muscle she has shown that there is a bundle of 2–4 axons per muscle fibre, surrounded by glia, twisting around each other (Fig. 5). There is a series of synapses along the length of the muscle fibre: from six to 37 synapses per nerve fibre, spaced at a mean distance of approximately 9 μm , but sometimes as much as 143 μm apart and often irregularly clumped. Reed (1995b) calculated the cable constant to be 552 μm , so that there seems to be a safety factor of approximately four times the largest synaptic interval. Presumably this allows graded, yet rapid, contractions of the radial muscle. However the mean number of synapses per nerve fibre (for both yellow and red chromatophores) is 22, so that there may be nearly 100 excitatory synapses on a single radial muscle (see Section II.4). Electron-lucent vesicles, approximately 50 nm in diameter, are present in all synapses; their size and appearance is consistent with their containing L-glutamate (L-glu), thought to be the excitatory transmitter of the chromatophores (see Section II.3). Immunohistochemical staining of the chromatophores with an antibody to L-glu at the light microscopic level stains the nerves positively (Fig. 4), and in the electron microscope the reactivity has been shown to be restricted to axons (Messenger *et al.*, 1997).

Sometimes there is a fibre in the nerve bundle that lacks such vesicles; instead it contains large, 90 μm diameter, electron-dense vesicles thought to contain serotonin (5-HT). There is positive staining of some

Table 1. *Size and density of chromatophores*

Species	<i>Loligo plei</i>	<i>Alloteuthis subulata</i>	<i>Lolliguncula brevis</i>	<i>Sepia officinalis</i>	<i>Octopus vulgaris</i>
Maximum diameter (μm)	120–1520	140–1350		300	300
Density (mm^{-2})	8	3	6	200–500 (hatchling) 35–50 (adult)	230

Based on data in Cornwell *et al.* (1997), Hanlon (1982), Hanlon & Messenger (1988), Packard & Sanders (1971).

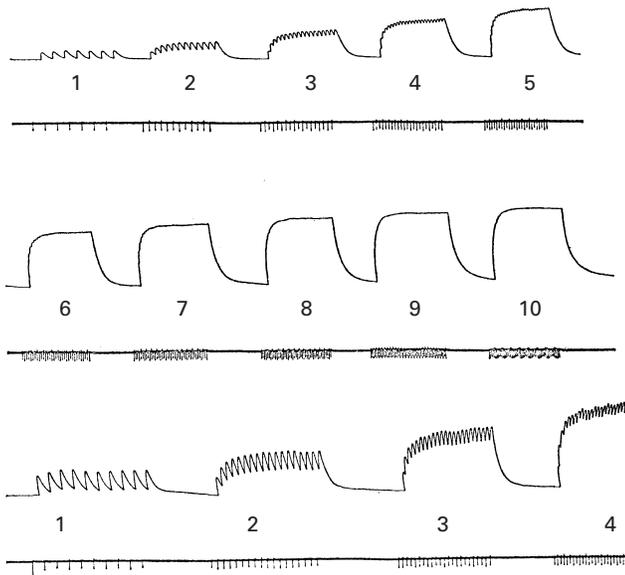


Fig. 6. Responses of the muscles fibres of a single chromatophore of *Loligo opalescens* to nerve stimulation at the frequencies (Hz) indicated. The lowest trace was recorded at higher amplification (Florey, 1966).

chromatophore nerves in the light microscope with an antibody to 5-HT (Messenger *et al.*, 1997). The vesicles are found along the whole length of the nerve but are never 'clumped' to form synapses (see Section II.4).

(d) Glial cells

These always accompany the axons; their processes lie beneath the external lamina of the muscle fibre and cover the outer surface of the axons as they proceed along the muscle fibre (Figs 1, 5).

(e) Chromatophore sheath cells

These conspicuous cells cover the chromatophores and their radial muscle, sometimes in four or more

layers; according to Cloney & Florey (1968) they are not coupled in any way, but according to Mirow (1972*a*) they are.

(f) Size and density of chromatophores

The size of the chromatophores varies according to species: the largest measured to date are found in loliginid squids (Table 1). The density of chromatophores also varies among different cephalopods. It is relatively low in epipelagic squids and very much higher in *Octopus vulgaris* and *Sepia officinalis* (Table 1). The size and density of chromatophores also vary in different regions of the body.

No quantitative data are available about the chromatophores of oceanic squids nor for any of the mesopelagic or bathypelagic forms, many of which have few chromatophores, sparsely distributed. For obvious reasons body patterns are simpler and bolder in cephalopods with fewer, larger chromatophores, and more subtle and refined in those with densely packed, small chromatophores, such as *Sepia* spp. and *Octopus* spp. (Section VI).

(2) Physiology

(a) Stimulation experiments

The cephalopod chromatophore is a neuromuscular organ, so that it is amenable to conventional physiological analysis. However, rather than recording radial muscle contractions mechanically, it has proved easier to use a photo-cell to record changes in the chromatophore in response to stimuli. Such a technique was developed long ago by Bozler (1930); more recently it has been modified by Florey (1966; Florey & Kriebel, 1969), working with the squid, *Loligo opalescens*. These workers used suction electrodes to stimulate nerve bundles in *in vitro*

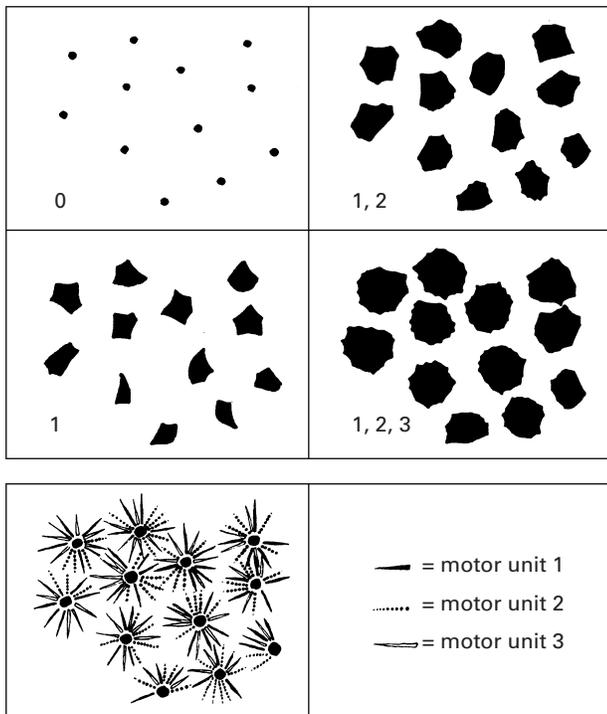


Fig. 7. Stepwise expansion of a set of brown chromatophores of *L. opalescens* as a result of recruitment of additional motor units. 0, unstimulated; 1, one axon; 1,2 two axons; 1,2,3 three axons stimulated (at 20 Hz). Lower boxes show distribution of motor units photographed during an experiment; only three motor units are shown (Florey, 1969).

preparations of skin and recorded the activity of all colour classes of chromatophore (brown, red and yellow in this species). Supra-threshold shocks of 0.5 ms were applied at increasing frequency with the following results.

(i) A single impulse elicited a twitch; at frequencies above 2 Hz there is considerable summation of contraction, and a smooth tetanus is usually achieved at frequencies above 10 Hz (Fig. 6). Maximum shortening and tension was obtained at 20–25 Hz.

(ii) With the chromatophores of the ventral mantle, which are large and sparsely distributed, increasing the voltage above threshold had no effect, suggesting that a single motor fibre innervates all the radial muscles of a chromatophore.

(iii) With the chromatophores of the dorsal mantle, which are small and numerous, increasing the voltage elicited a stepwise, increased expansion of the chromatophore, suggesting the recruitment of additional motor units (Fig. 7): the maximum number of steps obtained by Florey (1969) was six. Assuming there are 24 radial muscles per chromato-

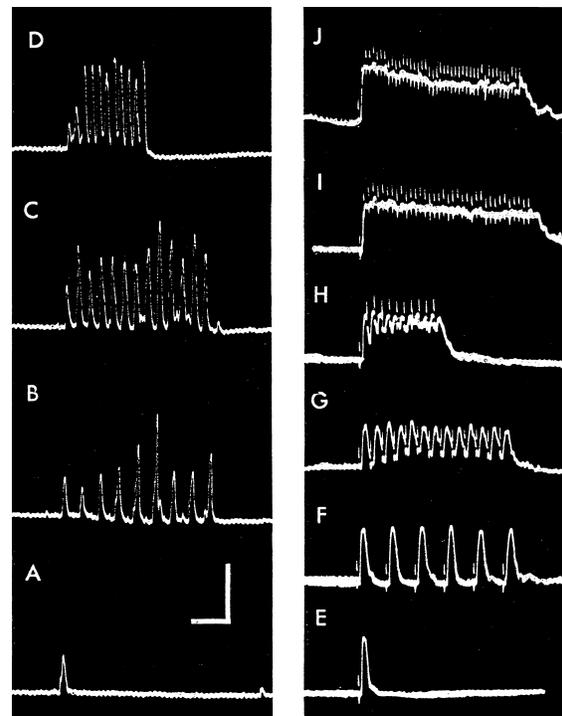


Fig. 8. Minimal facilitation (left panel) and absence of summation (right panel) of excitatory post-synaptic potentials during repetitive stimulation of a chromatophore motor nerve of *L. opalescens* at constant stimulus strength. Records A–D at 1, 16, 24 and 40 Hz, respectively; E–J (from different fibre) 1, 10, 24, 40, 64 and 80 Hz, respectively. No propagated spikes are elicited even at the highest frequencies. Calibration bars: 10 mV, 10 ms (Florey & Kriebel, 1969).

phore, there are no fewer than four motor units regulating its expansion. Thus the control of the chromatophores can be very precise, even in squids with their large chromatophores and simple body patterns (Section VI).

(iv) Differently coloured chromatophores were apparently innervated separately. Perhaps the most significant of these findings is that they include a direct physiological demonstration of the fact that in cephalopods many chromatophores may be innervated by more than one motoneuron, a significant point for pattern generation (see Section VI).

(b) Intracellular recording

Penetrating and ‘holding’ a chromatophore radial muscle with a glass microelectrode is not easy: they are not particularly narrow (approximately 7–13 μm , Reed, 1995*b*) but the whole chromatophore is free to move in the dermis. Nevertheless, Florey (1966) and Florey & Kriebel (1969) did succeed in making a few penetrations while stimulating the

chromatophore nerves, making the significant discovery that nervous stimulation is accompanied only by small, non-propagating excitatory post-synaptic potentials (EPSPs) (Fig. 8). The potentials showed neither facilitation nor summation, and it was impossible to elicit spike potentials. The local potentials could be recorded anywhere along the entire length of the muscle fibre and the inference from this that the muscle is polyterminally innervated has now been confirmed by the ultrastructural data of Reed (1995*b*) cited above. Florey & Kriebel (1969) also showed that increasing the stimulating voltage led to a stepwise change in EPSP amplitude; it was possible to record six or seven distinct steps, further evidence of polyneuronal innervation.

Recently Lima, Messenger & Brown (1997) succeeded in iontophoretically injecting the radial muscles with Calcium Green-5N and following cytoplasmic $[Ca^{2+}]$ changes during chromatophore expansion and retraction: specifically they showed that L-glu increases and 5-HT decreases cytoplasmic $[Ca^{2+}]$ (see below). Lima, Messenger & Brown (1998) also developed a method for dissociating the chromatophores and, working with an isolated radial muscle loaded with Fura-2 AM, found that glutamate-evoked $[Ca^{2+}]$ increase does not occur in Ca^{2+} -free artificial sea water.

Two other key findings from Florey & Kriebel's (1969) intracellular study need stressing. First, they were unable to find any evidence that the radial muscles receive inhibitory innervation (see Sections II.3c and II.4); and secondly they found that the chromatophores in ageing skin preparations reacted quite differently from those in fresh ones (see Section II.5). Such chromatophores often exhibit spontaneous muscle contractions and pulsate: intracellular recording reveals that such pulsations are caused by spike potentials, which are preceded by generator depolarisation. The electrical coupling of neighbouring muscle cells allows the spike to spread through the entire set of radial muscles of the chromatophore.

(3) Pharmacology

The pharmacology of the chromatophore organ has also received much attention, perhaps because certain substances, known to be transmitters elsewhere, produce such dazzling visual fireworks when applied topically to cephalopod skin (Fig. 9, Plate 2).

Currently three neuroactive substances are known to be implicated in the regulation of the chromatophores in different cephalopods: L-glutamate (L-glu) and FMRFamide-related peptides (FaRPs), which expand the chromatophores; and serotonin (5-HT), which retracts them.

(a) L-glutamate

The observations of Bone & Howarth (1980) were the first to hint at the identity of the excitatory transmitter at the chromatophore nerve muscle junction. They showed that L-glu expanded the chromatophores in *Sepia officinalis*, and in the squids *Loligo vulgaris* and *Alloteuthis subulata*. This finding was substantiated by Florey, Dubas & Hanlon (1985) (Fig. 10), who showed that L-glu, and its agonists, kainate and quisqualate, expanded the chromatophores of another loliginid squid, *Lolliguncula brevis*.

Subsequently Messenger and his collaborators (Messenger *et al.*, 1991, 1997; Cornwell & Messenger, 1995; Lima *et al.*, 1997, 1998) confirmed and extended these findings in the squids *Alloteuthis subulata* and *Loligo vulgaris* in four ways. First they tested the effects on the chromatophores of a whole range of specific glutamate agonists and antagonists recently developed by mammalian pharmacologists. The principal findings are summarised in Tables 2 and 3. It can be seen that the chromatophores expand (i.e. the radial muscles contract) when exposed to L-glu or any of its non-NMDA agonists: particularly effective is domoate, which in mammals is active at kainate/AMPA receptors. Similarly, they are sensitive to kainate/AMPA receptor antagonists,

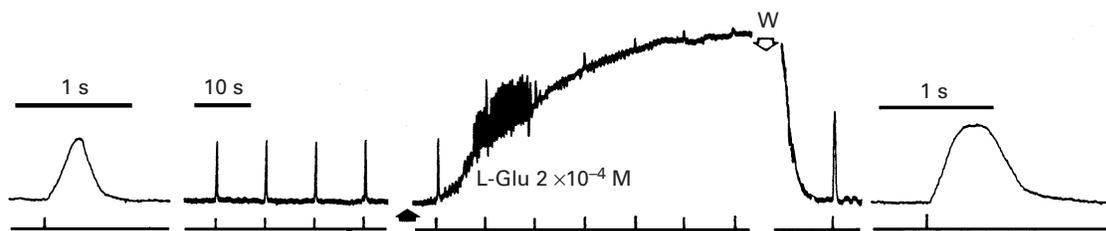


Fig. 10. *Lolliguncula brevis*: chromatophore muscle contraction (measured using a photocell) induced by L-glutamate. Note single twitches and enhancement and prolongation of twitches, reversed by washing (W) (Florey *et al.*, 1985).

Table 2. *Thresholds of some glutamate agonists that expand squid chromatophores*

Agonist	Threshold (mol l ⁻¹)
L-glutamate	1 × 10 ⁻⁴
AMPA	5 × 10 ⁻⁴
Kainate	1 × 10 ⁻⁴
Quisqualate	2 × 10 ⁻⁵
Domoate	5 × 10 ⁻⁷

AMPA, (RS)- α -amino-3-hydroxy-5-methyl-4-isoxasole propionic acid. Modified from Messenger *et al.* (1997). Data from *Loligo vulgaris* and *Alloteuthis subulata*.

Table 3. *Effects of some specific glutamate antagonists on squid chromatophores*

Antagonist	Concentration	Effect
AMPA/kainate		
CNQX	5 × 10 ⁻⁵ M	Expansion reversibly blocked
DNQX		
NMDA		
CPP	up to 5 × 10 ⁻⁴ M	No effect

AMPA, (RS)- α -amino-3-hydroxy-5-methyl-4-isoxasole propionic acid. CNQX, 6-cyano-7-nitroquinoxaline; DNQX 6, 7-dinitroquinoxaline. CPP, 3-[(RS)-2-carboxypiperazine-4-yl]-propyl-1-phosphoric acid. NMDA, N-Methyl-D-aspartic acid. Based on data in Messenger *et al.* (1997) from *Loligo vulgaris* and *Alloteuthis subulata*.

such as the quinoxalinediones, which reversibly block the action of L-glu. Secondly they demonstrated that L-glu or its agonists are active on

denervated chromatophores, showing that they act directly on the postsynaptic (muscle) membrane. Thirdly they obtained positive staining of the chromatophore nerves with an antibody to L-glu (Fig. 4B), and, by examining such preparations in the electron microscope, showed that the staining was restricted to the axons running along the radial muscles. Finally they made measurements of intracellular [Ca²⁺] using photometric techniques to measure the effects of L-glu directly (Lima *et al.*, 1997, 1998). By injecting the chromatophore radial muscle intracellularly with Ca²⁺-sensitive dyes such as Calcium Green-5N or Fura-2 AM they recorded photometrically the dramatic increase in cytoplasmic Ca²⁺ levels in the radial muscle as L-glu depolarises the post-synaptic membrane and the muscle contracts.

Further support for L-glu being the endogenous transmitter comes from the work of Loi & Tublitz (2000), who, in *Sepia officinalis*, showed that the effect of L-glu could be reversibly blocked by another specific glutamate blocker, Joro spider toxin (JSTX: Kawai *et al.*, 1983). More importantly they also found glutamate-like immunoreactivity in many cell bodies in the posterior chromatophore lobe in the brain (see Section V).

There is no evidence for any of the other 'classical' transmitters being active at the chromatophores, at least not in the species examined, and we may conclude that there is now overwhelming evidence for L-glu being the fast excitatory transmitter of the chromatophores in cuttlefish, loliginid squids and octopuses (Loi, Tublitz & Messenger, 1997). Students of the earlier literature will note that Florey and his colleagues had also examined the effects on

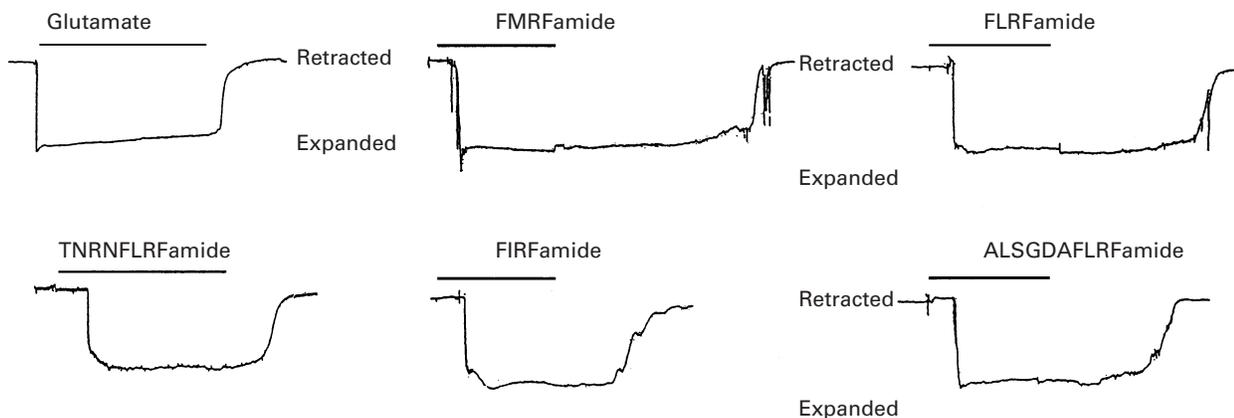


Fig. 11. Slow and prolonged expansion of chromatophores of *Sepia officinalis* after topical application of peptides. The bar above each trace indicates the period of transmitter application. This was 10 min, left column, and 5 min, centre and right columns (modified from Loi *et al.*, 1996, 1997).

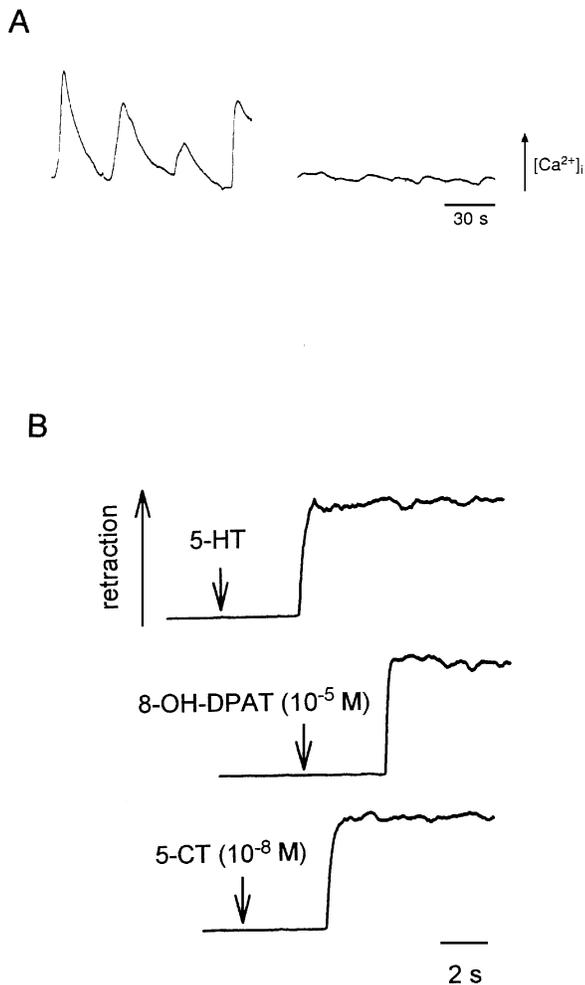


Fig. 12. (A) Abolition of 'spontaneous' calcium transients in *Loligo vulgaris* by $50 \mu\text{mol l}^{-1}$ 5-HT in a radial muscle fibre injected with Calcium Green-5N. Left, before application of 5-HT; right, 3 min after application. $[Ca^{2+}]_i$, cytoplasmic calcium concentration (Lima *et al.*, 1997). (B) Retraction of *Loligo bleekeri* chromatophores after application of serotonin (5-HT) or the serotonin agonists R(+)-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) and 5-carboxamidotryptamine (5-CT) (J. B. Messenger, unpublished data).

the chromatophores of acetylcholine (ACh), which had just been shown to be involved in regulating the anterior byssus retractor muscle of the bivalve, *Mytilus edulis* (Florey, 1966; Florey & Kriebel, 1969). In the light of recent evidence, we need only note how their careful experiments showed that, although ACh applied to the chromatophores elicited muscle contraction and chromatophore expansion, it had no effect on the resting potential of the radial muscle. They rightly assumed, therefore, that it must have been acting presynaptically to release the true, endogenous transmitter (L-glu), a finding confirmed much later by Messenger *et al.* (1997).

However, although L-glu seems to be primarily responsible for fast excitation (i.e. chromatophore expansion) in all cephalopods, it was shown recently that, in some, the chromatophores can be also be expanded by peptides.

(b) FMRFamide

In the cuttlefish, *Sepia officinalis*, Loi *et al.* (1996) have obtained positive staining in nerves running along the radial muscles with an antibody to FMRFamide, and have shown that the chromatophores can be expanded by several FMRFamide-related peptides (FaRPs) applied topically (Fig. 11). Three features of their results merit comment: (1) the response to the peptides is very much slower than that of L-glu; (2) recovery is also slow, as long as 4 min (recovery from L-glu is instantaneous, see Fig. 10); and (3) the threshold effective concentration in their *in vitro* preparation was sometimes as low as 10^{-9} mol l^{-1} (a typical value for L-glu is 10^{-4} mol l^{-1}). More recently Loi & Tublitz (2000), in the same species, showed that the FMRFamide almost certainly acts directly on the radial muscle, for it still elicits slow expansion of the chromatophores when the glutamate receptors have been blocked by JSTX.

It is significant that topically applied FMRFamide and other FaRPs fail to expand the chromatophores of five different species of loliginid squid, although they are active on the skin of octopods (Loi *et al.*, 1997). These facts have led to the hypothesis that those cephalopods that need to maintain their chromatophores tonically expanded during daylight hours for effective camouflage (Section VII) may utilize FaRPs for sustained patterning, while retaining L-glu as the transmitter for fast, transient colour change (Loi *et al.*, 1997; Loi & Tublitz, 2000). Confirmation of this hypothesis requires further data: it would be particularly interesting to have information about those loliginids, such as *Sepioteuthis sepioidea*, that show sustained body patterns for camouflage amongst corals (R. T. Hanlon, personal communication). Whatever the significance of the peptidergic regulation, however, it is indisputable that not all cephalopods control their chromatophores in precisely the same way.

(c) 5-HT

Kahr (1959) appears to have been the first to implicate 5-HT in chromatophore retraction in cephalopods, although he erroneously proposed that it was acting as a hormone. Florey (1966) and Florey

Table 4. *Effects on squid chromatophores of serotonin (5-HT) and its agonists and antagonists*

Receptor subtype		Drug	Threshold (mol l ⁻¹)	Effect
5-HT ₁	Agonists	5-HT	10	Retraction
		8-OH-DPAT	10 ⁻⁸	Retraction
		5-CT	10 ⁻⁸	Retraction
5-HT ₁	Antagonists	isamoltane	10 ⁻⁴	Slight expansion/ blocks 5-HT
		NAN-190	10 ⁻⁷	Slight expansion/ blocks 5-HT
		spiperone	10 ⁻⁶	Slight expansion/ blocks 5-HT
5-HT ₂	Agonist	α -Me-5-HT	10 ⁻⁴	Retraction
5-HT ₂	Antagonists	ketanserin	10 ⁻⁴	Blocks 5-HT
		mianserin	10 ⁻⁴	Blocks 5-HT
5-HT ₃	Agonists	2-Me-5-HT	10 ⁻⁴	No effect
		C144	10 ⁻⁴	No effect
5-HT ₃	Antagonist	LY 278, 584	10 ⁻⁵	Does not block 5-HT

8-OH-DPAT, R-(+)-8-hydroxy-2-dipropylaminotetralin; 5-CT, 5-carboxamido-tryptamine; NAN-190, 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl) piperazine; C144, 1-(m-chlorophenyl) biguanide; LY, 278,584: from RBI chemicals.

Data from *Loligo bleekeri* and *Alloteuthis subulata* (J. B. Messenger, unpublished data).

& Kriebel (1969) not only showed that 5-HT topically applied to the skin of the squid *Loligo opalescens* causes almost instant paling as the chromatophores retract, but established that 5-HT was not acting on the pigment cell itself, but on the radial muscles. Relaxation of the muscles enables forces stored in the cytoelastic pigment sac to retract the chromatophore. These workers also showed, by intracellular recording, that 5-HT has both pre-synaptic and post-synaptic effects. It does not interfere with synaptic transmission and does not affect the electrical properties of the post-synaptic (muscle) membrane, so that it cannot be regarded as a transmitter. Yet 5-HT does reduce the frequency of miniature post-synaptic potentials in the muscle (suggesting a pre-synaptic effect) and it increases the velocity of shortening and of relaxation of the radial muscles, implying an intracellular action within the muscle cell itself. Florey (1966, 1969) therefore suggested that 5-HT might exert its relaxing effect by moving Ca²⁺ into its stores within the cell.

The subsequent demonstration (Cornwell & Messenger, 1995; Messenger *et al.*, 1997) that 5-HT is actually endogenous in some of the chromatophore nerves, probably those with 90 nm vesicles (Section II.1c), made these early results of Florey even more significant. And Lima *et al.* (1997, 1998) have now obtained direct evidence to support the idea that 5-HT plays an essential part in chromatophore control and does so *via* Ca²⁺. After loading radial muscles with Ca²⁺-sensitive dyes they recorded dramatic decreases in cytoplasmic [Ca²⁺] in the presence of 5-

HT. 5-HT also blocks 'spontaneous' and caffeine-induced Ca²⁺ transients (Fig. 12A) and further experiments have shown that the Ca²⁺ stores are sensitive to ryanodine, though not to Pertussis toxin. It is apparent that 5-HT is acting at the chromatophore muscle by suppressing the release of Ca²⁺ from ryanodine-sensitive stores.

That the chromatophore muscles bear metabotropic 5-HT receptors has been shown in another way by Messenger *et al.* (1997). Even after denervation, squid chromatophores respond to 5-HT and certain 5-HT agonists by retracting. Table 4 lists the results of further unpublished experiments confirming that the radial muscles are sensitive to mammalian 5-HT₁ and 5-HT₂ agonists and antagonists (Fig. 12B), although not to agonists of the 5-HT₃ subtype, which is ionotropic (Walker & Holden-Dye, 1991; Boess & Martin 1994).

(4) Summary

We can now try to summarize the way in which the chromatophores are controlled in loliginid squids. Expansion is achieved by the activity of the excitatory nerves, which release L-glu from synapses along the length of the radial muscle. This leads to release of Ca²⁺ from ryanodine-sensitive stores in the sarcoplasmic reticulum (SR) and mobilisation of the contractile apparatus. The fact that transmitter release leads to non-propagated EPSPs ensures that chromatophore muscles can be activated individually. This allows for a delicate control of skin colour

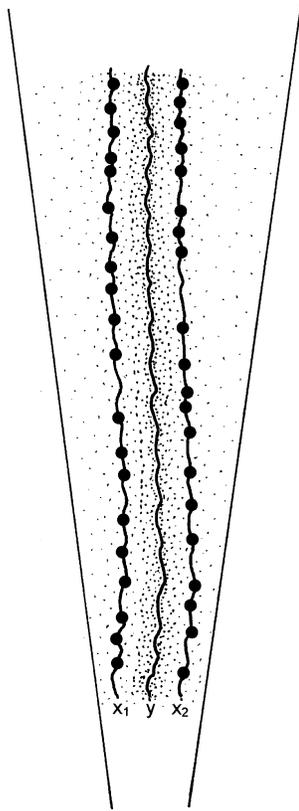


Fig. 13. Diagrammatic representation of a radial muscle fibre with three nerves (x_1 , x_2 , y); x_1 and x_2 are excitatory nerves that release L-glutamate at multiple synapses (●); y is the 'relaxing' nerve that releases serotonin (5HT) over its entire length (stippling) (based on data in Reed, 1995*b*).

by recruitment as well as by increasing the frequency of firing of the motor nerves. Under normal conditions there are never propagated potentials that cross the close junctions and activate neighbouring muscle fibres.

Retraction of the chromatophores is probably brought about mainly by contraction of the elastic sacculus, in the absence of activity in the excitatory motoneurons. However, the release of 5-HT, which suppresses the release of Ca^{2+} and mobilises its return to the SR, clearly facilitates the relaxation of the radial muscle. 5-HT is known to relax muscles in other molluscs, both gastropods and bivalves (Muneoka & Twarog, 1983; Walker & Holden-Dye, 1991), so its action in cephalopods is not unique. However, it needs emphasising that 5-HT is not acting as a neurotransmitter at the radial muscle: it has no effect on the post-synaptic membrane and it appears to derive from vesicles that are not grouped to form synapses, but are distributed along the entire length of the axon. Moreover, there is unequivocal

evidence that the 5-HT receptors on the radial muscle fibres are not ionotropic (5-HT₃-type) (Lima, 2000; J. B. Messenger unpublished observations) but are linked to G-proteins.

Why do cephalopods employ such a 5-HT system at the chromatophore muscles? Perhaps this is the most effective way of instantly switching off the excitatory effects of the L-glu emanating from nearly 100 synapses distributed along the length of the muscle among 4–5 separate nerves (Reed, 1995*b*; Fig. 13).

At this point it is necessary to introduce a major *caveat*. It is already known that octopod chromatophores are innervated quite differently from those of loliginid squids (Froesch, 1973*a*; Dubas, 1987); cuttlefish (and apparently some octopods) employ peptides as well as L-glu to regulate their chromatophores (Loi & Tublitz, 2000); and it is now becoming increasingly clear that the physiology of the different colour classes of chromatophore of the same species may differ slightly (A. Packard, personal communication). Thus caution must be exercised in generalising to all cephalopods results obtained from loliginid squids.

We should also make clear that cephalopod chromatophores are always assumed to lack a direct response to light (the so-called 'primary' response, common in many invertebrates, Weber, 1983). However, Packard & Brancato (1993) claim that, in *Octopus vulgaris* and *O. macropus*, light may act directly on the skin and this obviously merits further investigation.

(5) Chromatophore activity in the absence of nervous control

After death, the skin of a squid or octopus continues to show chromatophore activity for many hours, even days if the skin is kept cool. The spread of dark or pale waves across the skin is very striking: it was known to nineteenth-century physiologists, who named the phenomenon 'wandering clouds' (Wolkenwandern). These waves arise *post mortem* as the chromatophore nerves, but not the muscles, die; they have long been interpreted as evidence for some kind of 'network' linking the chromatophores, although the nature of such a network still eludes us.

In physiological preparations of fresh skin isolated chromatophores begin to pulsate as the preparation ages, all the radial muscles of a single chromatophore contracting in synchrony. As we have seen, Florey & Kriebel (1969) succeeded in recording from the radial muscles of pulsating chromatophores and

made the striking discovery that in such fibres there were propagated 'spikes' instead of the non-propagated EPSPs seen normally. Since neighbouring fibres are electrically coupled *via* the low-resistance close junctions the mechanism for synchronous pulsation of a single chromatophore is readily explained. How activity spreads from chromatophore to chromatophore through a population – as it undoubtedly does – is not understood, however. At one stage it was claimed that neighbouring chromatophores shared radial muscles (Froesch-Gaetzi & Froesch, 1977), which would obviously explain the linkage, but this claim has never been substantiated. Indeed there is good evidence to the contrary: Reed (1995*a*) succeeded in dye-filling over 200 radial muscles of squid chromatophores with Lucifer Yellow, none of which joined two chromatophores.

According to Packard (1992*a, b*, 1995*a, b*, in preparation), who has made a sustained study of the waves, other possible conducting elements for interchromatophore communication include the extracellular matrix or the dermis muscles; the phenomenon may perhaps involve field potentials. Whatever the mechanism involved, however, it is apparent that under what Packard (1995*a*) terms 'myogenic conditions', i.e. in the absence of nerve input, chromatophores, particularly chromatophores of the same type, begin to act in concert. As we shall see in Section III the chromatophores are vertically layered in the skin. In *Octopus vulgaris* the most superficial chromatophores are black; below them lie red, then orange then yellow chromatophores. This sequence is a developmental one, the yellows being the youngest, and the extraordinary feature of the wandering clouds is that they mainly, although not exclusively, pass through (i.e. link) populations of chromatophores of the same age/size/colour class. The waves that propagate through the chromatophores of a denervated area of skin may be dark (waves of contraction) or pale (waves of relaxation), but as the wave passes the chromatophores will always be brought to the same state of contraction. In short, they appear to be linked by a functional horizontal 'syncytium' (A. Packard, in preparation).

Experimentally one can induce waves in the short term by anaesthetizing normal skin (with ethanol or CO₂ but not urethane) or in the long term by denervating specific areas of skin, usually the mantle, and following changes in chromatophore behaviour over days or weeks. In long-term denervated *Octopus vulgaris* skin, for example, dark fast waves

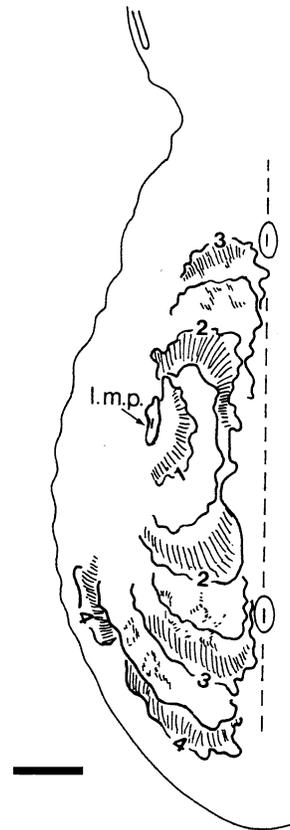


Fig. 14. 'Myogenic' propagation of waves across the skin of *Octopus vulgaris*. A fast, dark wave spreading across left-hand dorsal mantle skin from left long mantle papilla (l.m.p.) after long-term denervation. Darkening is indicated at four time points (1 s intervals). Note how the wave stops at the midline (dashed), where it meets the innervated skin of the intact right-hand side. Scale bar, 5 mm (modified from Packard, 1995*b*).

(10 mm s⁻¹; 0.2–1 Hz) can readily be observed emanating from a single point and following the same pathways as they spread through the skin (Fig. 14). Such fast waves do not arise instantly after denervation, however: initially there are slow waves (less than 1 mm s⁻¹) that persist for the first few days after lesioning, to be replaced by fast waves after a week or so (5–8 days according to temperature).

It needs emphasizing that wandering clouds do not form part of the normal chromatic repertoire of cephalopods in the sea: they are only likely to occur as a result of injury and any individual showing them would become most conspicuous to predators. They are essentially a *post mortem*, or experimentally induced, phenomenon. The interest in the waves lies in the evidence they provide for some, as yet unknown, system linking cells of similar age. The highly unusual cephalopod skin may thus prove an

interesting model for developmental studies (A. Packard, in preparation).

III. THE CHROMATOPHORE SYSTEM IN THE SKIN

So far we have been talking about isolated chromatophores, visible only to a biologist using a binocular microscope. In life, the chromatophores

operate in assemblies to be viewed by other animals, predators or conspecifics, and we must now consider the way in which groups of chromatophores are organized in the skin.

(1) The skin in three dimensions

A vertical section through the skin of a cephalopod reveals a surprisingly complex organization. Below the glass-like epidermis lie the chromatophores;

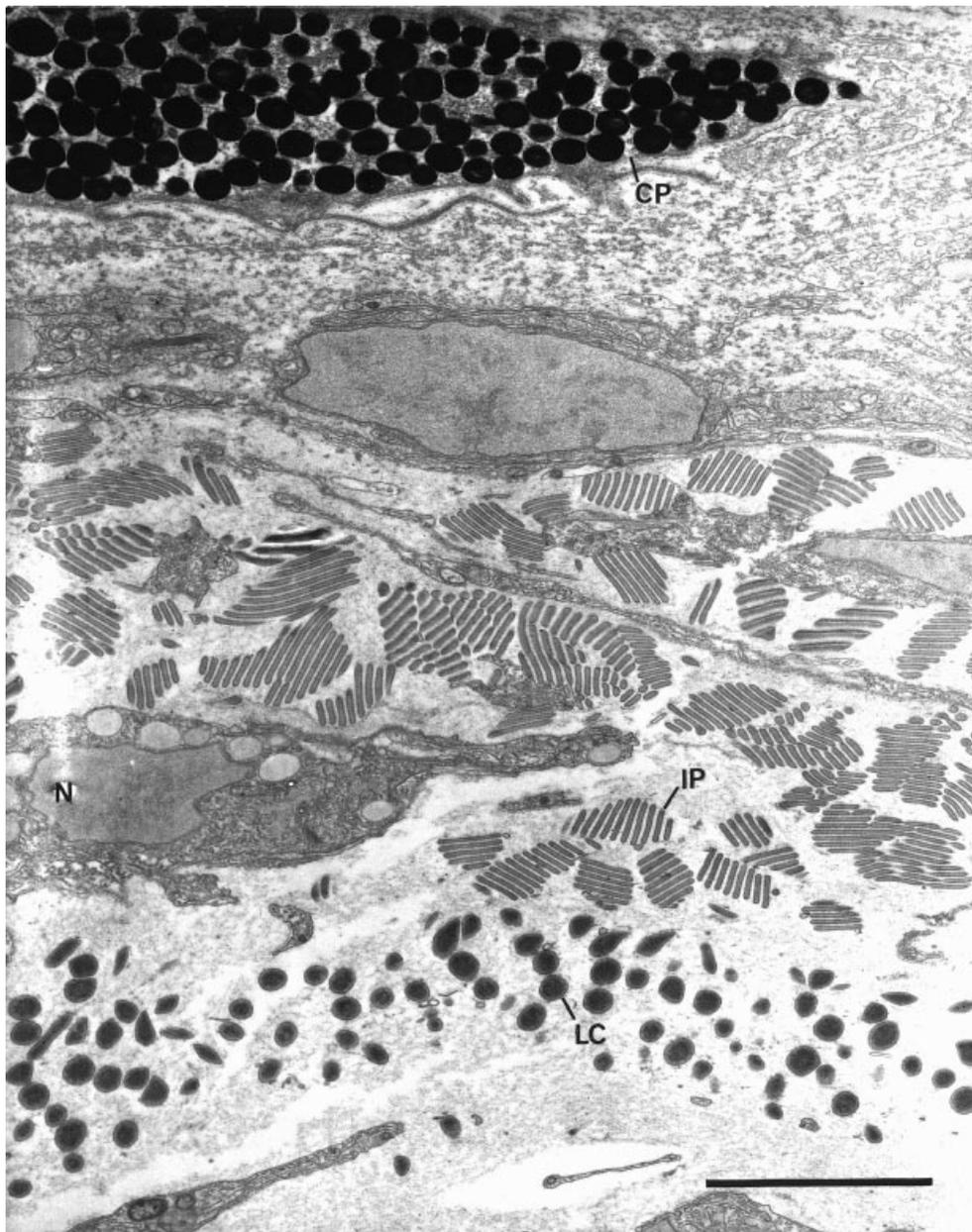


Fig. 15. Low-power electron micrograph of a vertical section through the skin in the head region of *Octopus vulgaris*, showing a superficial chromatophore (CP) above layers containing iridophores and leucophores. IP, iridosomal platelets; N nucleus; LC, leucophore clubs. Scale bar, 5 μm (Froesch & Messenger, 1978).

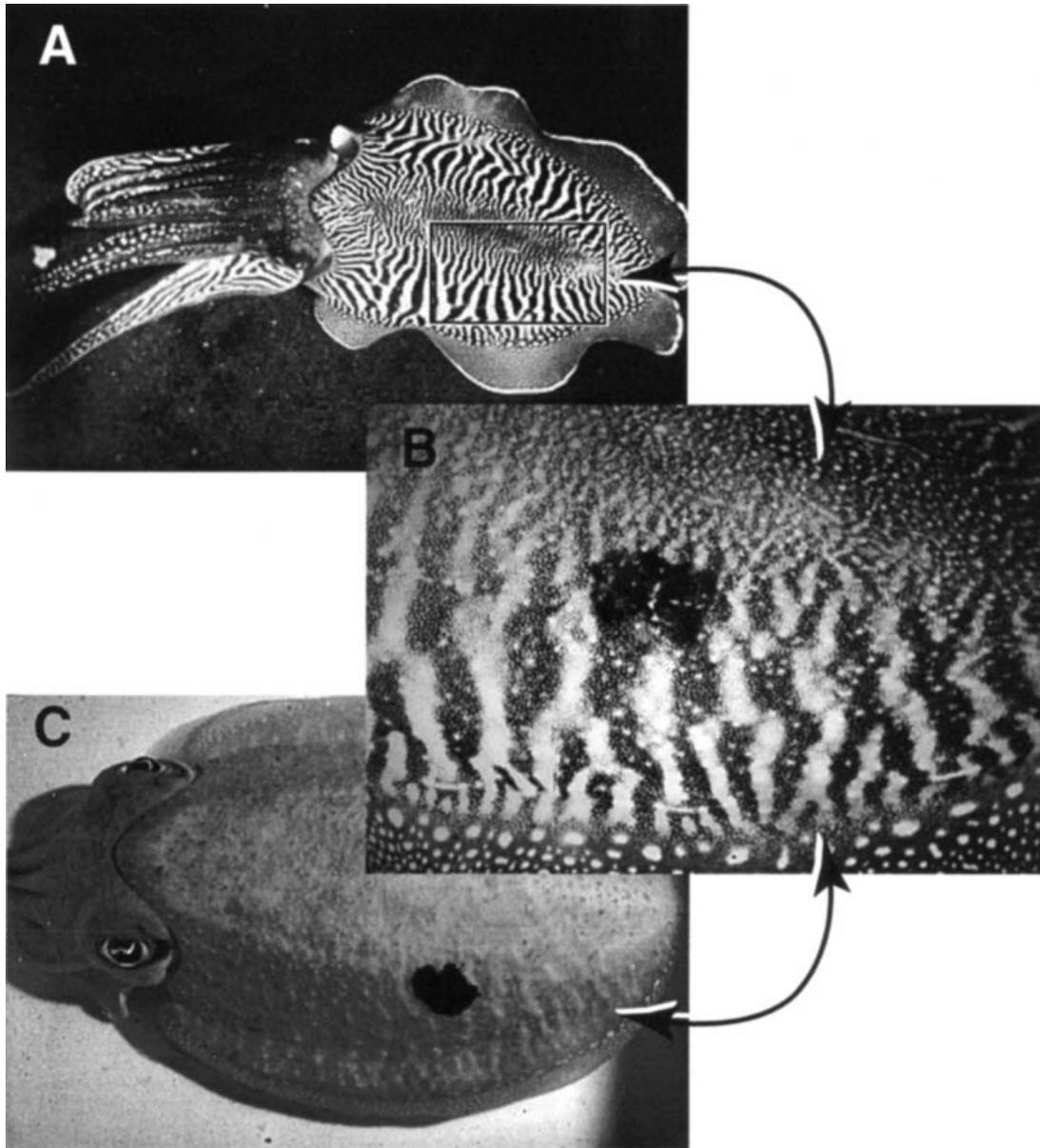


Fig. 17. A sequence showing how a particular group of chromatophores can participate in two different body patterns. A mature male cuttlefish (*Sepia officinalis*, mantle length approximately 150 mm) is showing the Intense Zebra body pattern in (A) and the Deimatic body pattern in (C). A photograph taken during the transition from one to the other (B) shows how the same dark chromatophores can contribute a zebra stripe or a warning spot.

below them are layers containing other 'elements' contributing to body patterning (Section VI. 2). The exact arrangement differs in different cephalopods, and in different regions of the body (see Packard & Hochberg, 1977; Hanlon, 1982). In the dorsal mantle skin of *Octopus vulgaris*, shown in Fig. 15, black chromatophores are the most superficial elements; below them are red chromatophores and deeper still the yellows. Below the chromatophores are the iridophores (producing blue-greens here)

and below these are the leucophores, broad-band reflectors.

A horizontal view of the same skin region (Fig. 16) makes plain that these various 'elements' are not randomly arranged: the iridophores occur across the entire area but the leucophores are clumped centrally, below the overlying chromatophores (Froesch & Messenger, 1978). Such a precise organization, which is presumably morphogenetically costly, suggests that strong selective

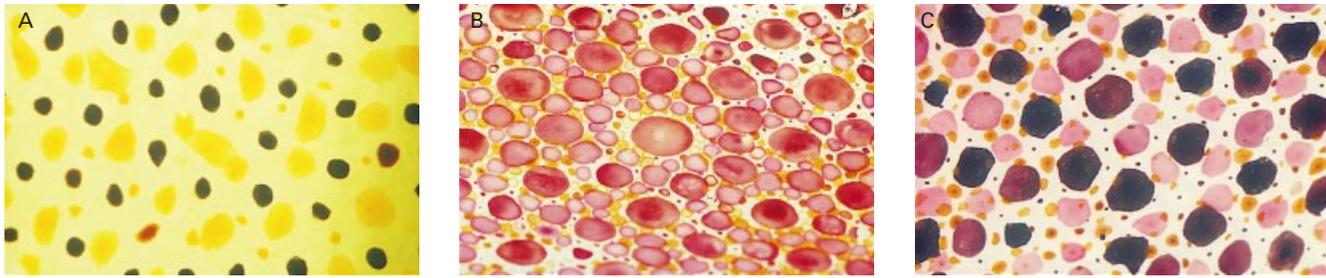


Fig. 2. The differently coloured chromatophores of three cephalopods: (A) *Sepia officinalis*; (B) *Loligo plei*; (C) *Sepioteuthis sepioidea*. Chromatophore pigments always reflect the longer waves of the spectrum, producing yellows, oranges, browns and reds; some are black. Magnification, A $\times 80$; B $\times 4$; C $\times 5$ (photographs courtesy of R. T. Hanlon).

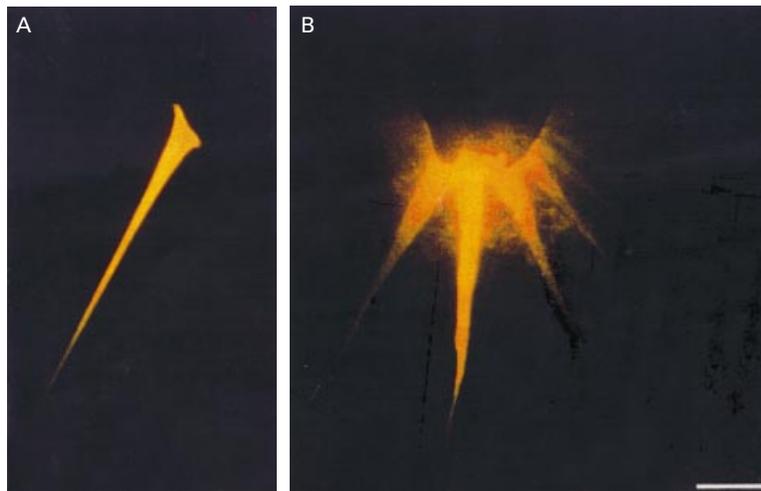


Fig. 3. Lucifer Yellow fills of (A) a single, uncoupled radial muscle fibre and (B) a group of dye-coupled muscle fibres in *Loligo vulgaris*. Scale bar, 0.2 mm (Reed, 1995a).

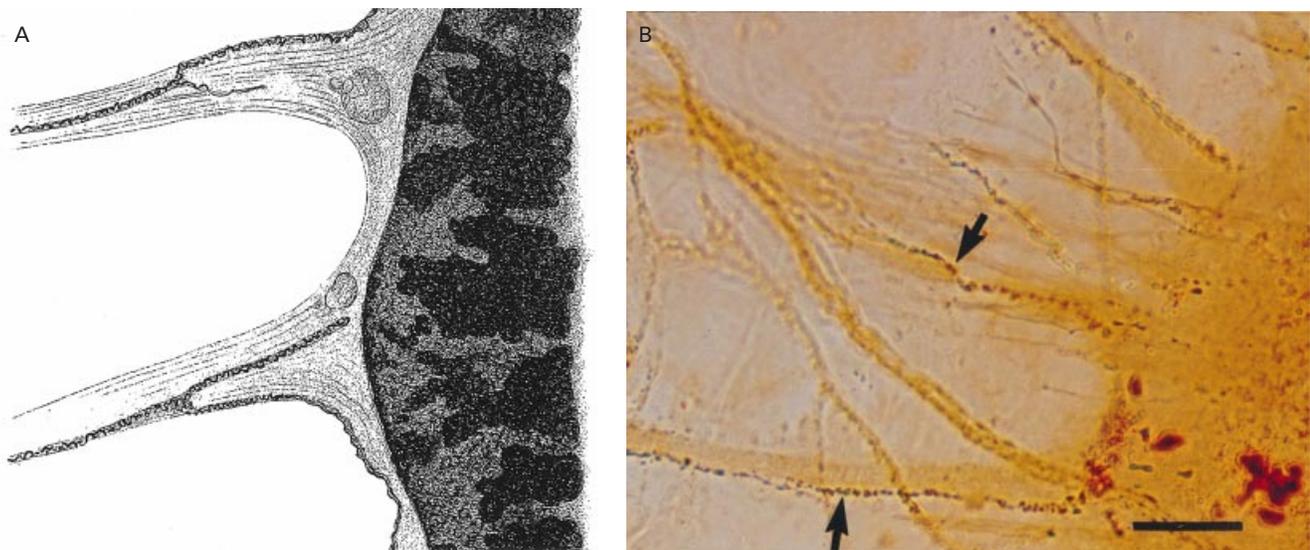


Fig. 4. Chromatophore nerves stained (A) with Methylene Blue (*Loligo vulgaris*; Hoffmann, 1907b) and (B) with L-glutamate antiserum and peroxidase-antiperoxidase/diaminobenzidine (*Alloteuthis subulata*; Messenger *et al.*, 1997). Axons marked with arrows; scale bar, 1 mm.

pressures must have been exerted during the designing of the octopus skin. (Section VIII). It is commonly said that the chromatophores represent a two-dimensional display on the skin of neural activity in the brain: we must remember, however, that the display is strictly a shallow, three-dimensional one.

(2) Morphological and physiological units: chromatomotor fields

It is convenient, although not without difficulties, to classify groups of chromatophores in the skin into 'morphological' and 'physiological' units (Packard & Hochberg, 1977; Packard, 1982). The first can be thought of as a static array in the skin, the second as a dynamic event, the result of neurally activating some elements in the array but not others. The fact that a single chromatophore receives multiple innervation is of critical importance here for it means that a particular chromatophore (or group of chromatophores) can participate in different patterns. One example of this is shown in Fig. 17, which shows two different physiological units acting *via* the same morphological units. As Packard (1982) puts it:

The arrangement of individual chromatophores in the skin is as much anatomically fixed as are the positions of light bulbs in an [illuminated] bill-board ... but the patterns seen are transient phenomena that result from the various ways in which the chromatophore elements are switched on It would be foolish to try to account for displays on the [bill-board] by giving a detailed description of the two-dimensional matrix of light bulbs making up the bill-board when such displays are a function of spatio-temporal connections encoded in a central programme. And yet a description of the matrix is necessary if one wants to account for the quality of the pictures displayed ... the grain, the colours.

To study morphological units, then, it is appropriate to record (by photograph or video camera) the appearance of the living animal in order to detail the precise arrangement of chromatophores (iridophores, reflector cells and leucophores) in various parts of the body (e.g. Hanlon & Messenger, 1988). To study physiological units one can also rely on photographs; but it is relatively easy to elicit such units by direct electrical stimulation.

If a stimulating electrode is placed on the skin of an anaesthetized or freshly dead cephalopod the chromatophores will expand locally, revealing what Packard (1974) terms a 'motor field', or a 'chro-

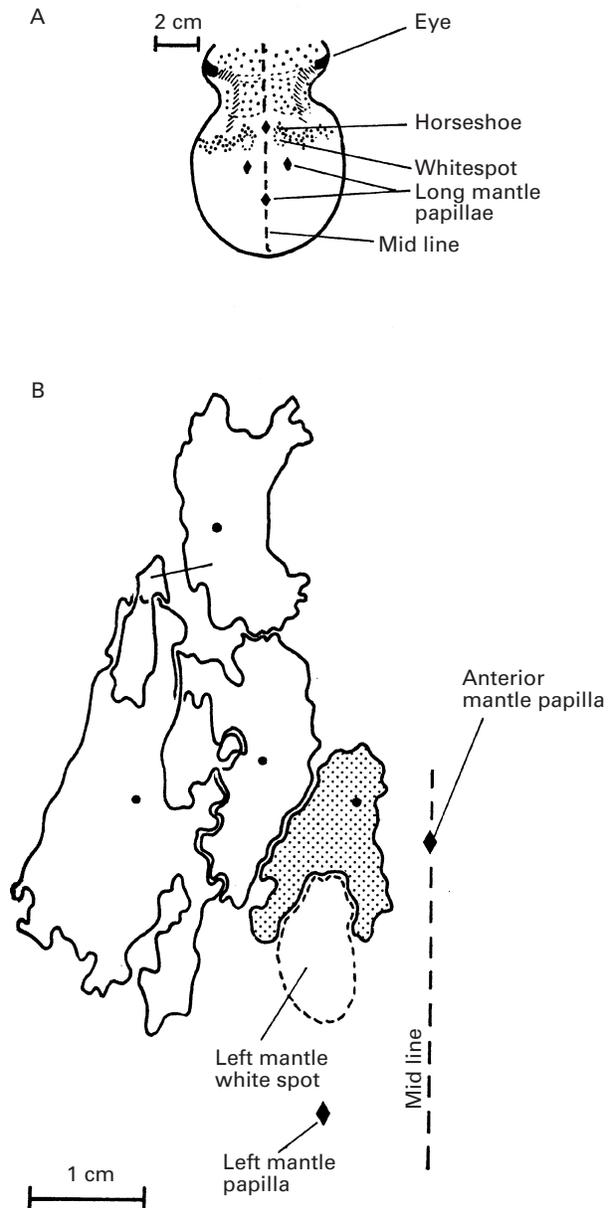


Fig. 18. Chromatomotor fields of *Octopus vulgaris* (A) Dorsal view of the head and mantle showing some components of body patterns. (B) Enlargement of part of (A), showing tracings of the fields of expanded chromatophores produced by direct electrical stimulation at the points shown (●). Note that the four chromatomotor fields shown have complementary or partially overlapping boundaries, and that the horseshoe (stipple) is complementary to the white mantle spot (modified from Packard, 1974).

matophore field' but which, following Demski (1992), we propose to call a chromatomotor field. It is noteworthy that such a field is rarely circular: instead it is usually highly irregular (Fig. 18) and if the stimulating electrode is moved within that

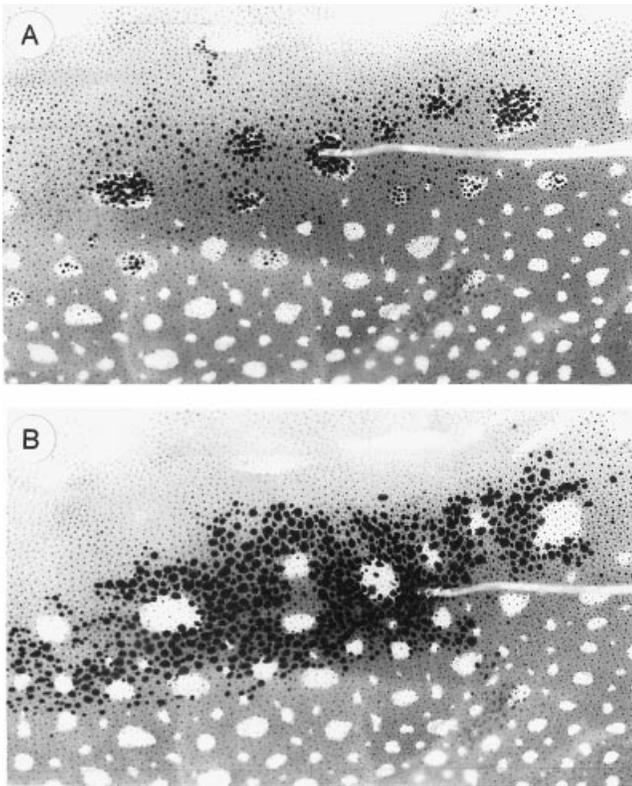


Fig. 19. The white fin spots of the cuttlefish *Sepia officinalis* can be (A) obliterated (as in concealment) or (B) enhanced (as in signalling), by exciting the appropriate motoneurons with a silver electrode held on the skin. Scale bar, 3 mm (Hanlon & Messenger, 1988).

chromatomotor field there is very little change in its appearance. However, if the electrode is moved outside the field a new chromatomotor field will appear, equally irregular, whose boundary is complementary to the adjacent fields. The significant point is that such fields, which persist long after death or after section of the chromatophore nerves, are part of the natural patterning of a living octopus. Similar fields can be elicited in cuttlefish skin: Fig. 19 shows two kinds of elongate field that conceal or enhance the fin white spots (for camouflage or signalling respectively).

Such chromatomotor fields depend upon the result of the distribution of chromatophores in the skin and the organization of motor units, in the classical sense of that term (Maynard, 1967; Florey, 1969; Packard, 1974, 1983; Packard & Hochberg, 1977). Presumably the stimulating electrode placed on the skin is activating the terminal branches of a particular chromatophore motoneuron (similar results are obtained by stimulating nerve bundles: Dubas & Boyle, 1985), but that neuron is expressing its activity through the way the chromatophores it

innervates are spatially distributed in the skin. Maynard (1967) was the first to recognize this, and point out that cephalopod skin in some ways functions as a 'retina in reverse': he coined the term 'pattern-position separation' to describe the way the geometry of effector (or receptor) elements peripherally is related to the central neurons driving (or responding to) them.

One way out of the apparent difficulty of reconciling morphological and physiological units has been suggested by Packard (1982), who points out that we need to consider the development of patterning during ontogeny. As new chromatophores arise in the skin and new chromatophore motoneurons are recruited into the brain their outgrowing fibres must make contact with the newly available radial muscles of the newcomers. The motoneurons will thus connect with chromatophores of the same age-class (and therefore size and colour) spread across more than one morphological unit. They can thus be thought of as 'chronological units' (or 'chronomers'), which 'successively intersect the morphological array as it develops' (Packard, 1982). We shall return to this point in Section IV (see Fig. 27).

(3) Mapping the chromatomotor fields

Since the chromatophores are innervated directly from the brain it should be possible to map the projection of chromatophore nerves onto the body surface and this has been done, at least at a gross level. Selective lesioning of the chromatophore nerves in *Octopus vulgaris*, which leaves pale, denervated areas on the skin, has shown that chromatophore fibres are carried in 10 nerves leaving the brain on each side (Fig. 20A). The whole body is thus divided into 20 areas, some very small (iris), some large (individual arms); the two largest areas are on the mantle, where each side is supplied by fibres running in the pallial nerve (Froesch, 1973b).

It has been possible to continue the mapping a little further in the mantle. Many years ago Sereni & Young (1932) showed that chromatophore fibres from the brain to the mantle run in the pallial nerve to the stellate ganglion, through which they pass without synapsing to leave *via* some 40 stellar nerves to run to the periphery (Fig. 20B). Bühler *et al.* (1975) electrically stimulated each of the stellar nerves in turn and recorded the results photographically. Fig. 20C, which summarizes their findings, shows how the chromatophore nerves are distributed around the mantle in a regular way.

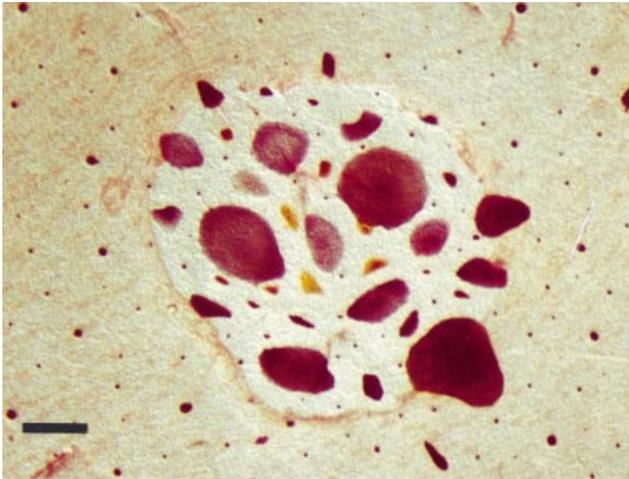


Fig. 9. The effect of topical application of L-glutamate ($5 \times 10^{-4} \text{ mol l}^{-1}$) to *Alloteuthis subulata* skin (mounted upside down with a circular window cut in the dermis). Scale bar, 1 mm (Messenger *et al.*, 1997).

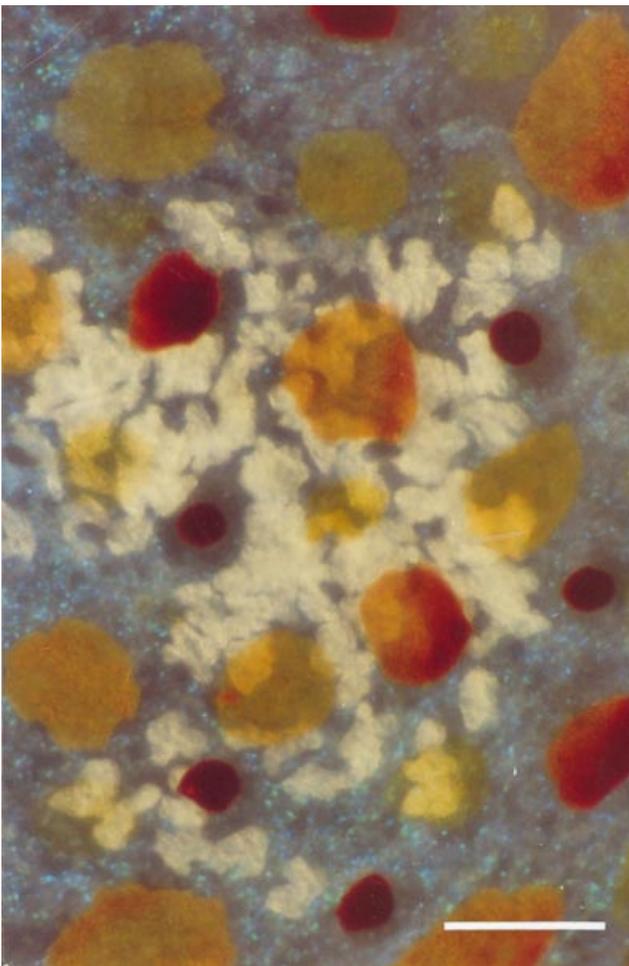


Fig. 16. A circular chromatic unit in the arm skin of *Octopus vulgaris*. Note the different coloured chromatophores lying above a group of leucophores, reflecting white, and the numerous small iridophores, which appear blue-green at this angle of viewing (electronic flash). Scale bar, 50 μm (Froesch & Messenger, 1978).

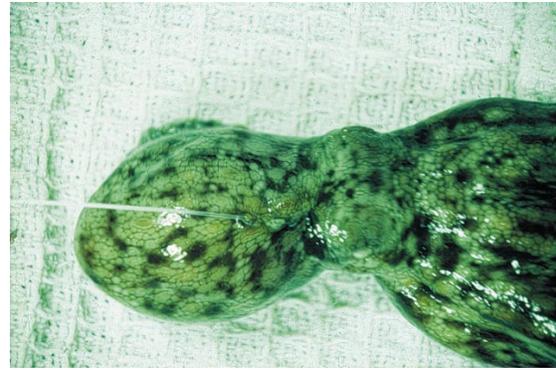


Fig. 33. Anaesthetised *Octopus vulgaris* (mantle length, 80 mm) after injection of serotonin ($10 \mu\text{g}$ in $100 \mu\text{l}$ sea water) into the cephalic aorta. The patterning resembles the mottling shown by unrestrained octopuses in conflict situations (Andrews *et al.*, 1981).



Fig. 40. The countershading reflex: when a lightly anaesthetized cuttlefish *Sepia officinalis* (mantle length, 110 mm) is held at 90° in the roll plane the chromatophores on the upper half of the entire ventral body surface all expand (Ferguson *et al.*, 1994).



Fig. 41. *Octopus zonatus* (mantle length, 30 mm). Disruptive pattern (Hanlon, 1988).

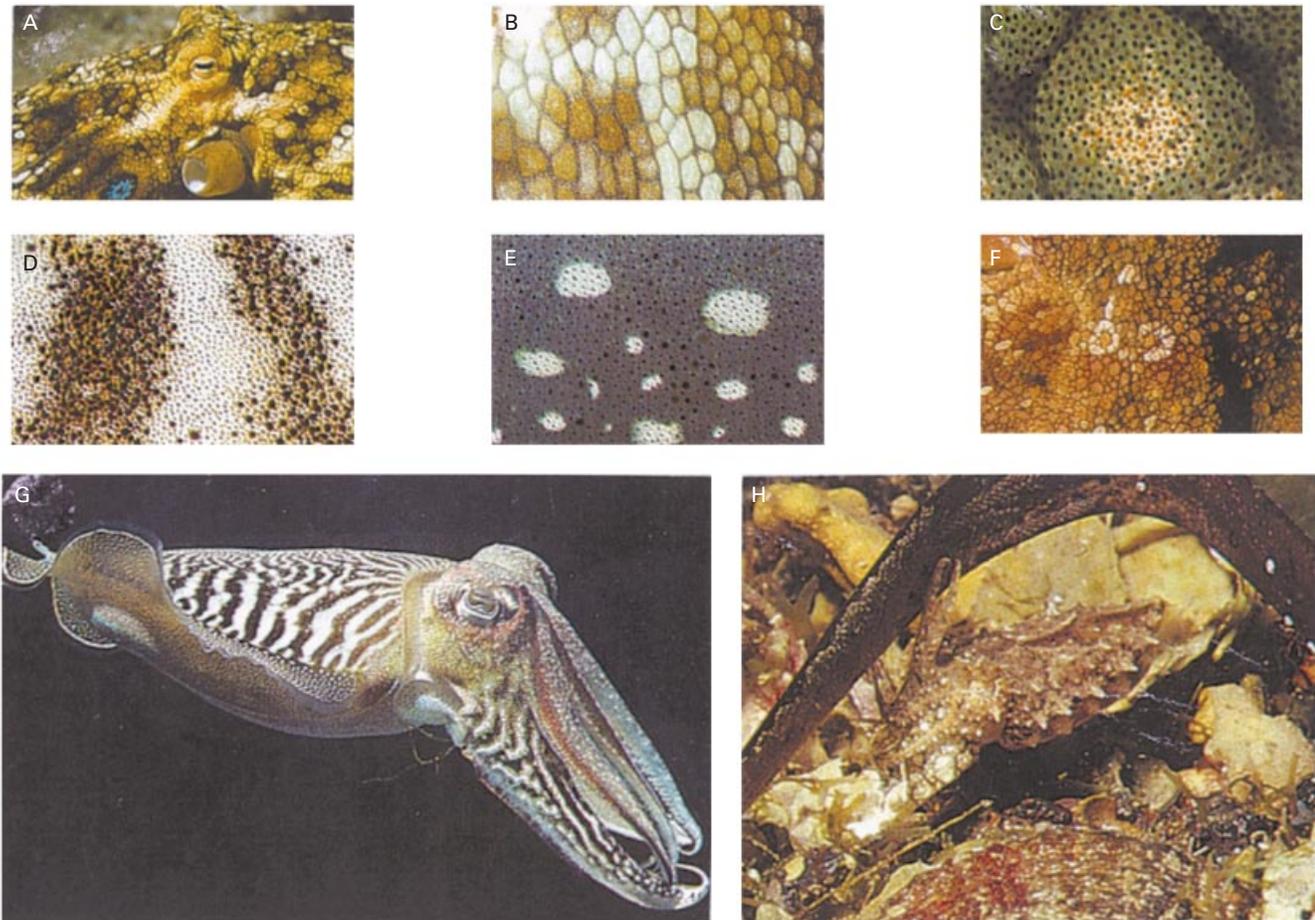


Fig. 36. Living cephalopods. (A) *Octopus bimaculatus*: blue iridescent eye-spot (mantle length, c. 65 mm); (B) *O. burryi*: patch and groove unit in mantle skin ($\times 3$); (C) *O. vulgaris*: similar unit with central leucophores reflecting white ($\times 3$); (D) *Sepia officinalis*: zebra stripes, produced by chromatophores and leucophores ($\times 3.5$); (E) *S. officinalis*, fin white spots, produced by leucophores ($\times 4.5$); (F) *O. bimaculatus*: frontal white spots (leucophores) whose effect is disruptive or 'epistreptic' ($\times 1$); (G) *S. officinalis*: mature male displaying Intense Zebra (mantle length 180 mm); (H) *S. officinalis*: hatchling concealing itself with combination of chromatic, textural and postural components. Note Major lateral papillae and Raised arms (mantle length 10 mm). A–F, Hanlon & Messenger (1996); G–H, Hanlon & Messenger (1988).

Plate 3

There has to be some doubt about the sharp delineation claimed by these workers for each of these large chromatomotor fields, however. In a comparable, but much more thorough, study in the squid, *Lolliguncula brevis*, Ferguson *et al.* (1988) demonstrated substantial overlap between chromatomotor fields (Fig. 21). Indeed Bühler *et al.* (1975) themselves report overlapping fields on occasion but ascribe it to current spread. Certainly, where individual nerve fibres have been stimulated to elicit chromatomotor fields overlap seems the norm; for example, Dubas (1987) found chromatomotor fields with quite large overlap in the dorsal skin of *Eledone cirrhosa*, as did Packard (1974) in *Octopus vulgaris* (see Fig. 18). And in both *O. vulgaris* and *Loligo* spp.

(Packard, 1991, 1995a) there is a large overlap of innervation in the dorsal midline, the 'paramedian area', where overlapping control, from fibres in the left and right pallial nerves, may provide an essential safety factor for maintaining camouflage.

The fact that the chromatophore fibres from the brain to the mantle are clearly distributed *via* the stellar nerves in an organized, regular way has been demonstrated in another way by the experiments of Sanders & Young (1974). In *Octopus vulgaris* they crushed the pallial nerve unilaterally, partially denervating populations of chromatophores on that side of the mantle. They then allowed the damaged nerves to regenerate over periods of several months and showed that normal patterning could be re-

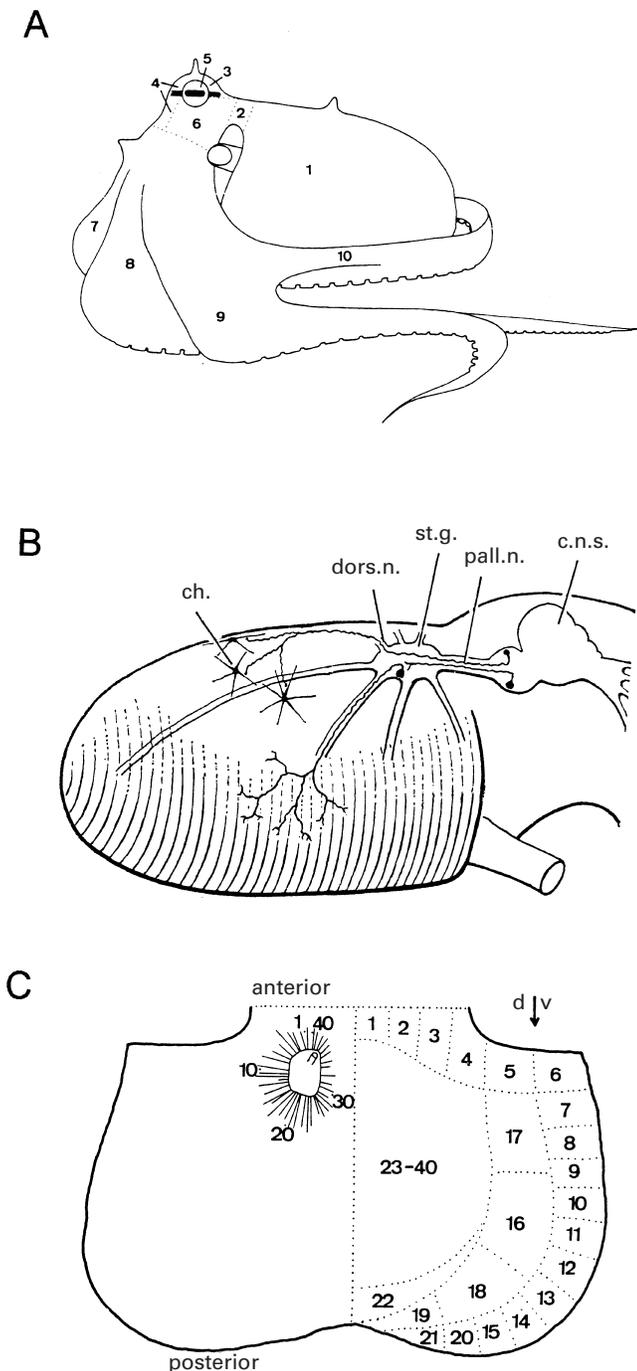


Fig. 20. (A) Projection of chromatophore motor fibres onto the skin of *Octopus vulgaris* (left side). Nerves indicated by the numbers are: 1, pallial; 2, collar; 3, posterior superior ophthalmic; 4, superior antorbital; 5, median superior ophthalmic; 6, anterior oculomotor; 7–10 arm nerves I–IV (Froesch, 1973*b*). (B) Innervation of the chromatophores of the mantle (ch.) of *O. vulgaris* by fibres whose cell bodies lie in the brain (c.n.s.): their axons run along the pallial nerve (pall. n.), pass through the stellate ganglion (st. g.) without synapsing, and leave *via* one of the dorsal stellar nerves (dors. n.). The mantle muscle, by contrast, is innervated indirectly, *via* motoneurons located

established, i.e. individual chromatophore nerves grow back to their original destinations. However these findings, reminiscent of regeneration in the amphibian visual system (Gaze, 1970), have never been followed up.

If the chromatophores are distributed in such a precise way – if there is a ‘map’ in the mantle – we might expect there to be an isomorphic map among motoneurons in the brain, specifically in the posterior chromatophore lobes (PCLs). This seems not to be true, however. The best evidence for this comes from the critical experiments of Dubas *et al.* (1986*a*) on *Lolliguncula brevis*, a squid with simple body patterns based on only nine chromatic components (Section VI). They injected horseradish-peroxidase (HRP) at different sites in the mantle skin and allowed retrograde transport (over the course of a week in the living squid) to carry it along chromatophore nerve fibres to motoneurons in the brain. They also used focal stimulation of PCL neurons in semi-intact preparations to elicit chromatomotor fields on the mantle. Their results are summarized in Fig. 22. The HRP injections reveal that there is no clear relation between injection site on the mantle or fin, and motoneuron location in the PCL. Dubas, Leonard & Hanlon (1986*b*) confirmed these findings, in addition showing that some chromatophore motoneurons for the mantle lie outside the PCL (see Section V.1*a*). Similarly, there is no relation between the loci of stimulation in the PCL and the regions of chromatophore expansion on the mantle (Fig. 22). This appears to be true for *Octopus vulgaris* as well (J. A. Miyan & J. B. Messenger, unpublished observations).

These findings are perhaps counterintuitive. Surely there ought to be a simple isomorphic map in the brain? It is worth recalling that, in *Sepia officinalis*, Boycott (1961), admittedly working with acute preparations, obtained some evidence for a crude topographical arrangement of chromatophore motoneurons in the PCL: mechanical stimulation of the

in the stellate ganglion. (Sanders & Young, 1974). (C) Diagram showing the distribution of chromatophore fibres to the different parts of the mantle of *O. vulgaris* *via* the stellate nerves, based on stimulation experiments. There are approximately 40 nerves leaving the stellate ganglion (left-hand side): fibres in these nerves control chromatophores in the areas shown on the right. Note that the mapping appears to be fairly regular, but that there is no information about the innervation of the dorsal mantle (see text). d ↓ v, border between dorsal and ventral parts of mantle (Bühler *et al.*, 1975).

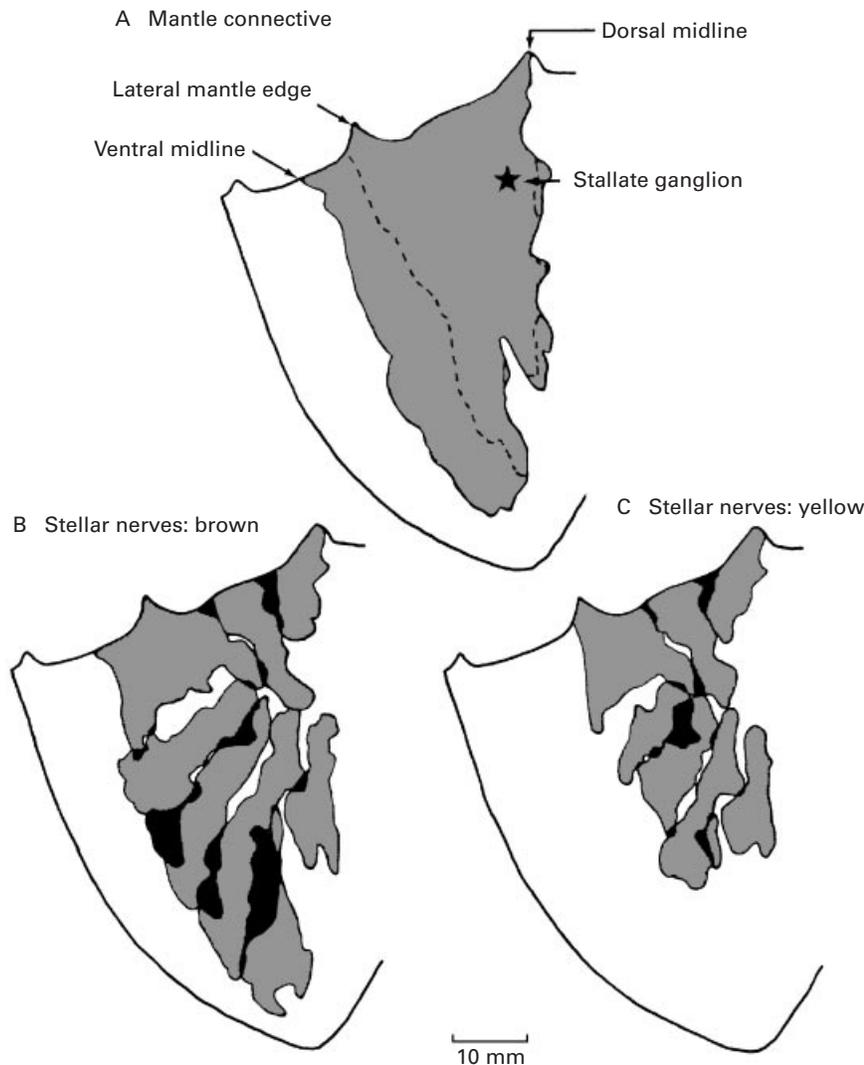


Fig. 21. Chromatomotor fields in *Lolliguncula brevis*. (A) Stimulation of the pallial nerve (mantle connective) expands brown and yellow chromatophores over the entire half-mantle of a squid; dashed line indicates limit of yellow chromatophores. (B, C). composite tracings of chromatomotor fields of fibres in seven stellar nerves (1–7). Note the overlap between the adjacent fields of both brown (B) and yellow (C) chromatophores (Ferguson *et al.*, 1988).

anterior and posterior parts of the PCL elicited chromatophore responses in the anterior and posterior parts of the mantle respectively. The answer may relate to ontogeny: Packard (1995*b*) argues that a particular 'area of skin houses many generations of chromatophore and many components one on top of another, and most components ... are a distributed global category tightly coordinated centrally'. Unfortunately, although it is clear that new chromatophores arise in the skin in between existing ones (Section IV), nothing is known about the development of new chromatophore motoneurons in the brain. Do they arise in layers adjacent to the neuropil (see Fig. 29) or between established motoneurons? This is a problem meriting urgent examination.

(4) Recording from chromatophore nerves

Only two studies have documented chromatophore nerve activity in cephalopods during colour change. In a semi-intact preparation of *Lolliguncula brevis* Dubas *et al.* (1986*a*) placed cuff-electrodes around the pallial nerve and recorded activity during spontaneous chromatophore activity on the mantle and after extracellular stimulation of the PCL. In living *Sepia officinalis* Messenger & Miyan (1986) succeeded in attaching a suction electrode to the small bundle of chromatophore nerves running just below the skin to the 'deimatic spot' on the mantle (Fig. 23). Using this preparation they were able either to stimulate the nerve, thus generating the spot, or to record from it while eliciting the spot by

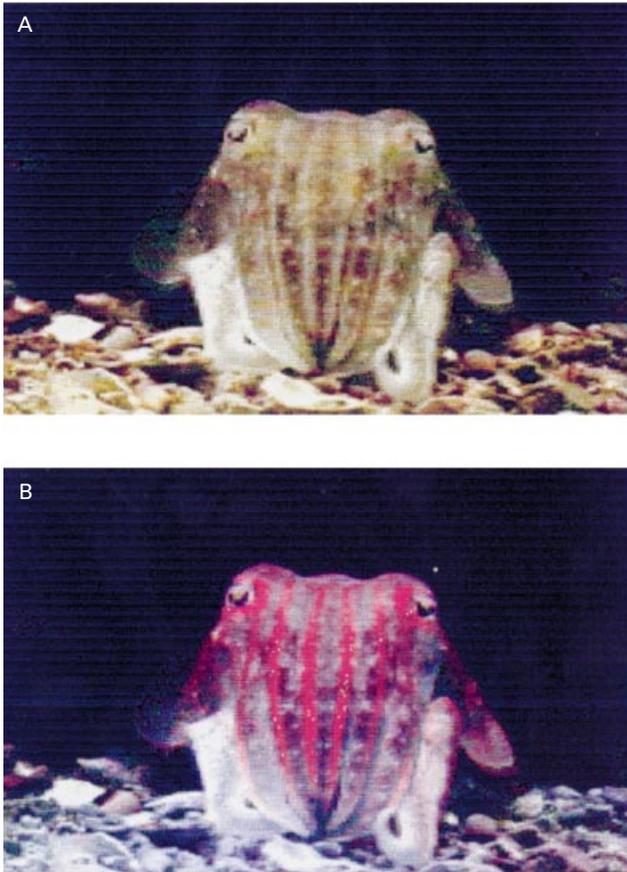


Fig. 44. (A) Full-colour image of an adult cuttlefish, *Sepia officinalis*, seen head on. (B) False colour image of same. In (B) the orientation of polarisation is coded to hue (horizontal, red); the percentage of polarisation is coded as saturation (full saturation represents total polarisation); and the intensity of the reflected light is coded as lightness. There is a clear pattern of stripes over the head and arms reflecting horizontally polarised light (Shashar *et al.*, 1996).



Fig. 45. Simultaneous dual signals: *Sepioteuthis sepioidea* (male, mantle length 100 mm) signals 'stay near' to a female on its right and 'keep away' to a male on its left (Hanlon & Messenger, 1996).

Plate 4

'frightening' the animal by waving a hand at it. There was a marked increase in activity in the nerve immediately preceding the appearance of the spot. Similar recordings from 'surround' areas near the spot showed decrease in firing rate as the surrounding chromatophores paled to enhance the blackness of the warning spot.

This is, to date, the only experiment in which chromatophore nerve activity has been recorded in a living cephalopod during 'natural' stimulation *via* the eye. It shows, rather surprisingly, very high

frequencies of firing in the chromatophore nerves generating the deimatic spot in the cuttlefish: the initial activity in some single units approached 100 Hz, as opposed to the 20 Hz found necessary for smooth tetanus by both Florey (1966) and Dubas *et al.* (1986a) in their squid preparations.

IV. ONTOGENY

The development of the chromatophores has been studied by a number of workers, notably Naef (1921,

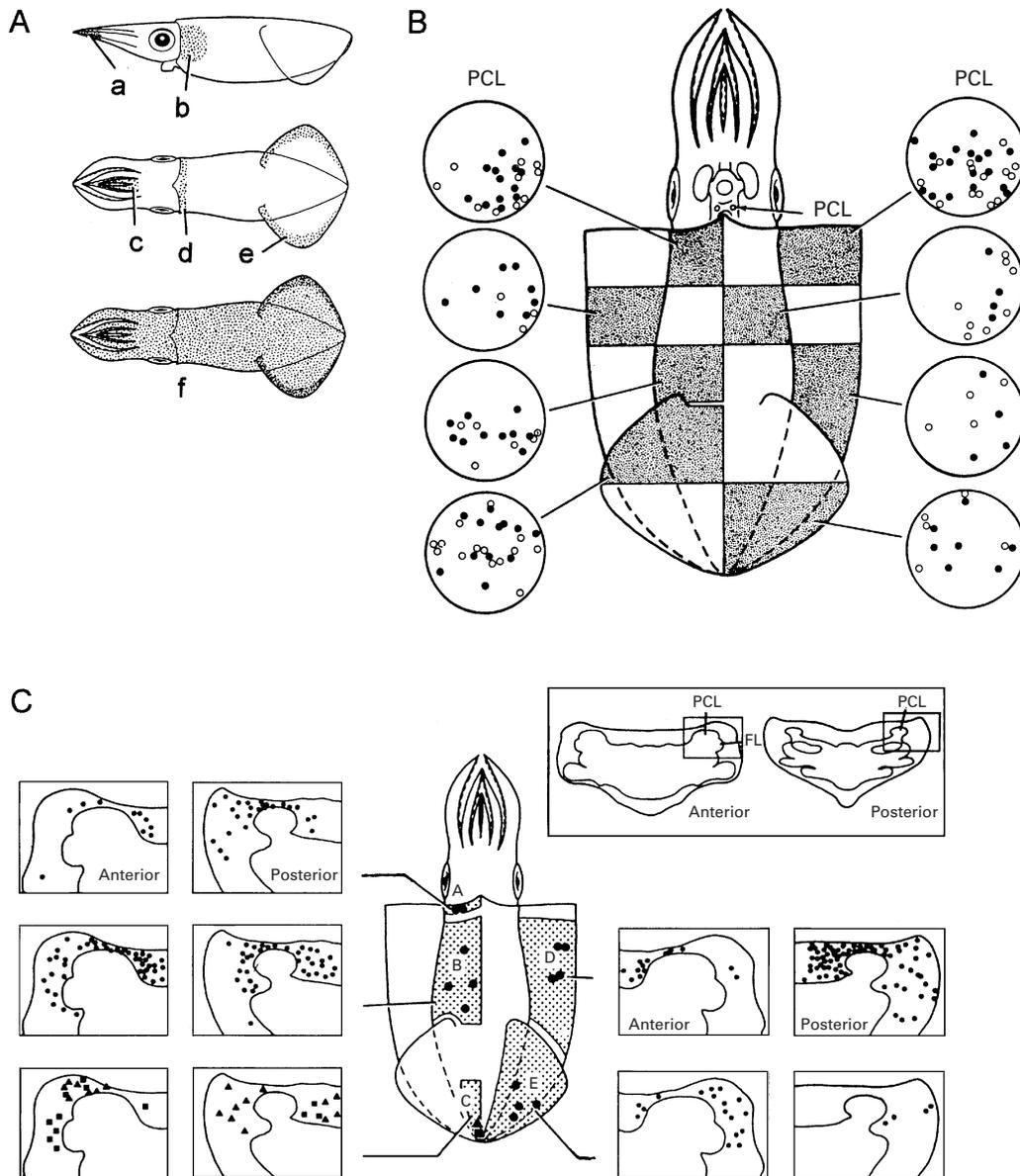


Fig. 22. (A) Some of the very simple chromatic components of body patterns of *Lolliguncula brevis*. a, Dark Arm Tips; b, Lateral Mantle Spot; c, Dark First Arms; d, Mantle Margin Stripe; e, Dark Fin Line; f, All Dark. (B) Lack of topographical relationship between the locus of stimulation in the posterior chromatophore lobe (PCL) and the region of chromatophore activation in the mantle skin. The mantle and fin are divided into eight areas on each side. Shaded areas indicate areas within which a motor unit response could be elicited by focal threshold stimulation on the surface of the ipsilateral PCL (shown in the eight large circles) with monopolar (●) or bipolar (○) electrodes. (C) The complementary experiment: retrograde transport of horseradish peroxidase from sites in the mantle skin to the brain. Again there is no clear relationship between the location on the body and the location in the PCL. Injections were made in five areas of mantle (A–E): motoneurons were found mainly in the PCL, but also in the fin lobe (FL) (Dubas *et al.*, 1986a).

1928) and Fioroni (1965; Poggel & Fioroni, 1986), but it is Packard (1982, 1985, 1991) who has highlighted the importance of ontogenetic studies in understanding the functional organization of the chromatophore system in the skin of adult cephalopods.

In the squid *Loligo vulgaris*, the detailed ultrastructural study of Poggel & Fioroni (1986) has shown that the first chromatophores appear fairly late in embryogenesis: hatching occurs at Stage XX of Naef, but chromatophores cannot be distinguished properly until Stage XIII. The various cell types

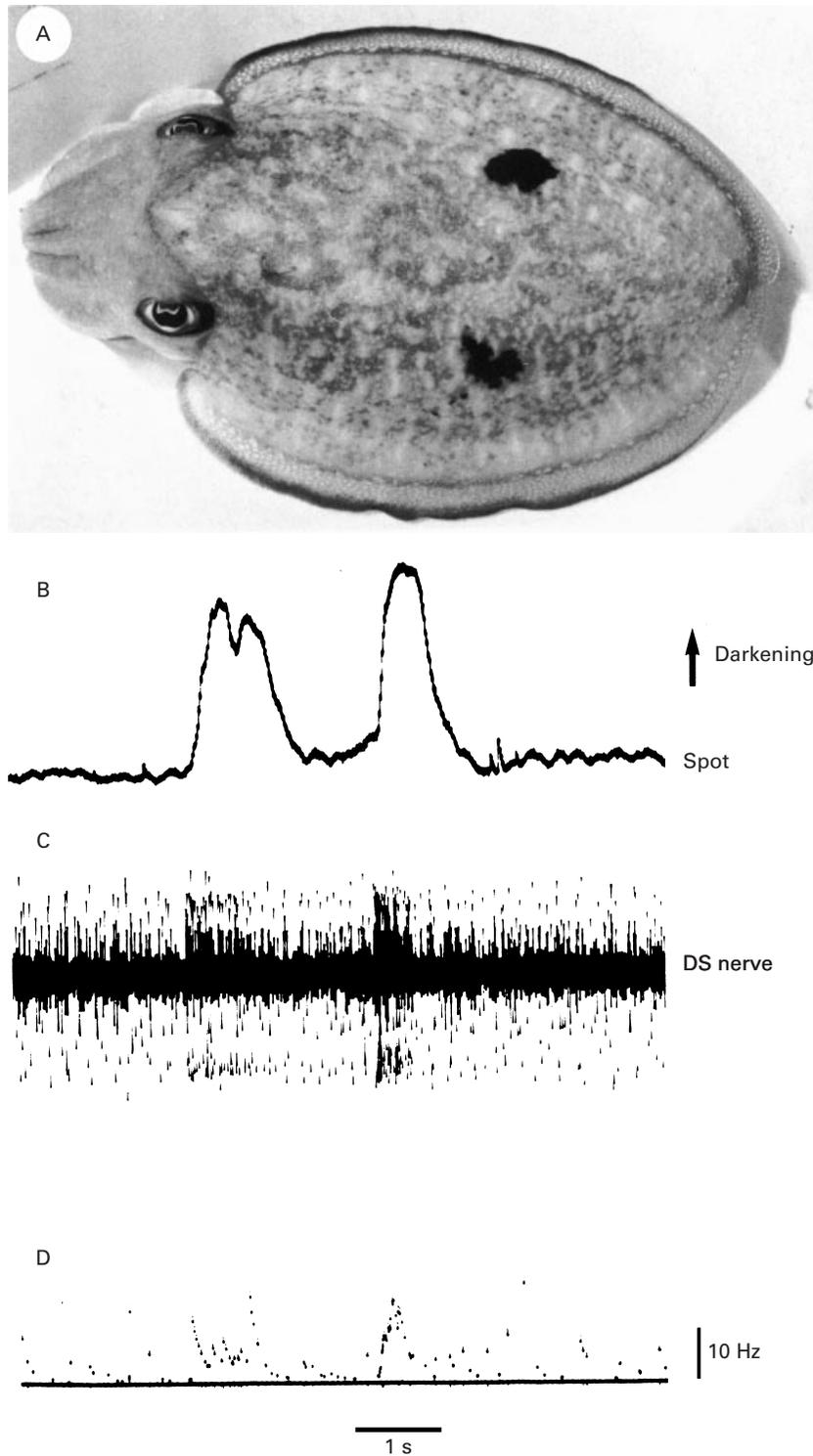


Fig. 23. (A) An adult cuttlefish (*Sepia officinalis*, mantle length 120 mm) showing the Deimatic body pattern, whose most conspicuous feature is the pair of large dark eyespots. (B)–(D) Simultaneous records of (B) eyespot darkening as spot is displayed twice (recorded by video-densitometry), (C) changes in activity of eye spot nerve (DS) and (D) instantaneous frequencies of the largest single units in the DS nerve (Messenger & Miyan, 1986).

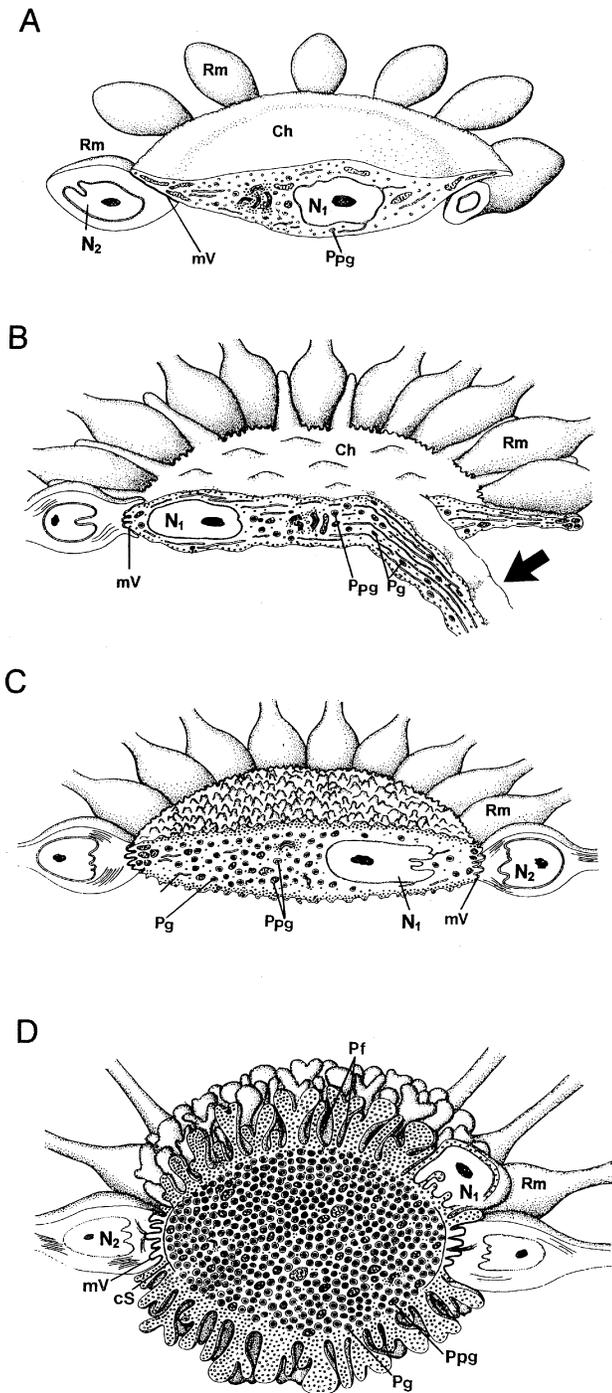


Fig. 24. Embryonic development of chromatophores in *Loligo vulgaris* (reconstructions based on electron microscopy: nerves and glia omitted). (A) Stage XIII–XIV with several radial muscle cells (Rm) lying close to the central chromatophore cell (Ch). (B) Stage XIV–XV with (left) the muscles beginning to attach (mV) to the central cell and (right) an enlargement of one of the processes extending between the muscles (arrow). (C) Stage XV–XVI, with unbroken contact between the muscle cells and the central cell (note its increased pigmentation). (D) Stage XVI–XVII, with the ap-

pearance of these organs then appear, develop and interact between Stages XIV and Stage XVII (Fig. 24), by which time the chromatophore is virtually indistinguishable from the adult organ described by Cloney & Florey (Fig. 1). In *Loligo vulgaris* the chromatophores contract sporadically at Stage XVII and they appear to be functional by Stage XVIII, but unfortunately, Poggel & Fioroni (1986) do not give any details of the innervation nor of the way in which the nerves make functional contact with the chromatophore muscles during development. This is an important issue that has not yet been investigated in any cephalopod.

The nature of the chromatophores at hatching varies according to the hatchlings' life style. For example, in epipelagic squids and octopuses the chromatophores are few and sparse, for transparency is of prime importance for camouflage in the water column. In bottom-dwelling cuttlefish, however, the density of chromatophores on the dorsal mantle is almost 10 times greater in hatchlings than in adults (Hanlon & Messenger, 1988), for these animals rely on their complex body patterns to conceal themselves from sharp-eyed predators.

In cephalopods with relatively few chromatophores at hatching, such as loliginid squids, it has proved possible to use the chromatophores for taxonomy, because their distribution is sufficiently distinct and characteristic to permit classification down to species level (McConathy, Hanlon & Hixon, 1980: Fig. 25). Useful though this fact may be for systematists, it depends upon a fundamental feature of the chromatophores that has profound significance for any understanding of their organization in the adult animal: the spatial organization of the chromatophores is never random. It is always patterned (Packard, 1985).

The chromatophores are organized in four 'tegumental fields': arms, head, mantle and funnel, each one of which contains conspicuous 'founder chromatophores' (Fig. 26). These fields persist throughout life. Each has its own polarity and rate of chromatophore genesis, and those on the arms and

pearance of the cytoelastic sac Anlage (cS), near the myochromatophoral junction (mV), and the deep primary infoldings (Pf) of the chromatophore surface. N₁, N₂, nuclei of chromatophore central cell and radial muscle, respectively; Pg, pigment granule; Ppg, propigment granule. Simplified from Poggel & Fioroni (1986); cf. Fig. 1.

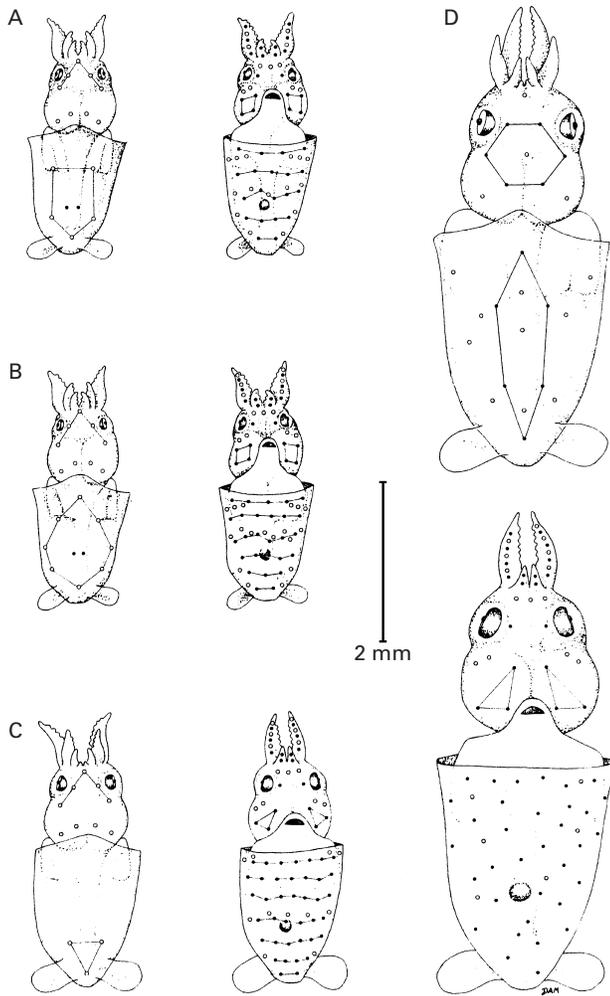


Fig. 25. Diagrammatic representation of the arrangement of chromatophores in four species of loliginid squid hatchlings (1–5 days). (A) *Loligo plei*, (B) *L. pealei*, (C) *Lolliguncula brevis* (dorsal aspect on the left, ventral on right); (D) *Loligo opalescens* (dorsal uppermost). All to same scale. Dots, retracted reds; circles, retracted yellows (McConathy *et al.*, 1980).

mantle have been explored in detail by Packard (1985). This important paper also describes a set of simple ‘rules’ for the development of the chromatophore system during post-embryonic life. The rules were obtained by careful observation of hatchling *Octopus vulgaris* and by following changes in the same area of skin from the same individual over time. They may be summarized thus: (1) extant (older) chromatophores retain their position and never disappear; (2) new chromatophores arise in spaces between extant ones (evidently as a result of lateral inhibition: Meinhardt & Gierer, 1974); (3) new (younger) chromatophores are smaller than older ones and remain so, thus creating an age/size hierarchy; (4) new chromatophores are yellow: they darken with age, becoming orange, then red, then brown (although apparently not all of them: Packard, 1990).

These rules can be followed to some extent in Fig. 26, and their consequences can be followed in Figure 27. This shows how the morphological and physiological units discussed in Section III are related: the units can be seen as a succession of developmental classes, that is they are chronological units, or ‘chromomers’ as Packard (1982) styled them. After the new chromatophores arise they will attract innervation from motoneurons in the brain and the shape of the new units thus formed will depend on the positions of the previous generations of chromatophores. Unfortunately, however, we know nothing about the details of the new innervation: indeed, we do not know how and where the new chromatophore motoneurons arise in the brain (Section V).

It is also worth recalling that during development the chromatophores are not only influenced by other chromatophores, but by other ‘elements’ in the skin, such as leucophores (Section VI). In adult *Sepia*

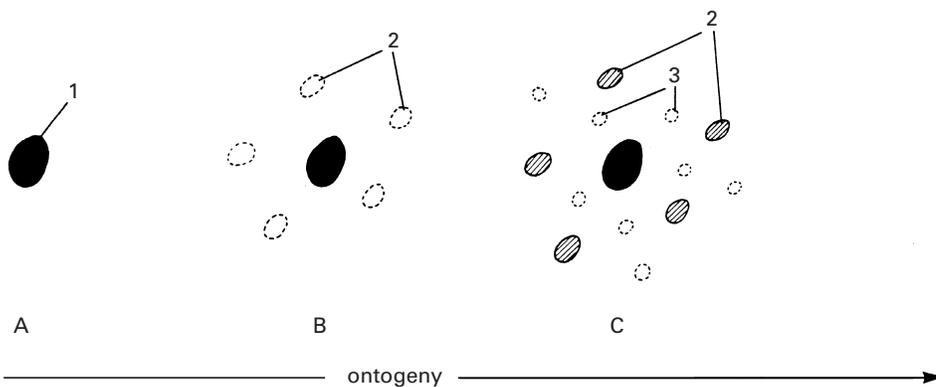


Fig. 26. Epigenesis of chromatophores in young *Octopus vulgaris*. (A) A single founder chromatophore (black – 1). (B) Five new, smaller, second-generation chromatophores – 2. (C) Eight (still smaller) third-generation chromatophores – 3. Yellow chromatophores, open; orange ones, hatched (Packard, 1988*a*).

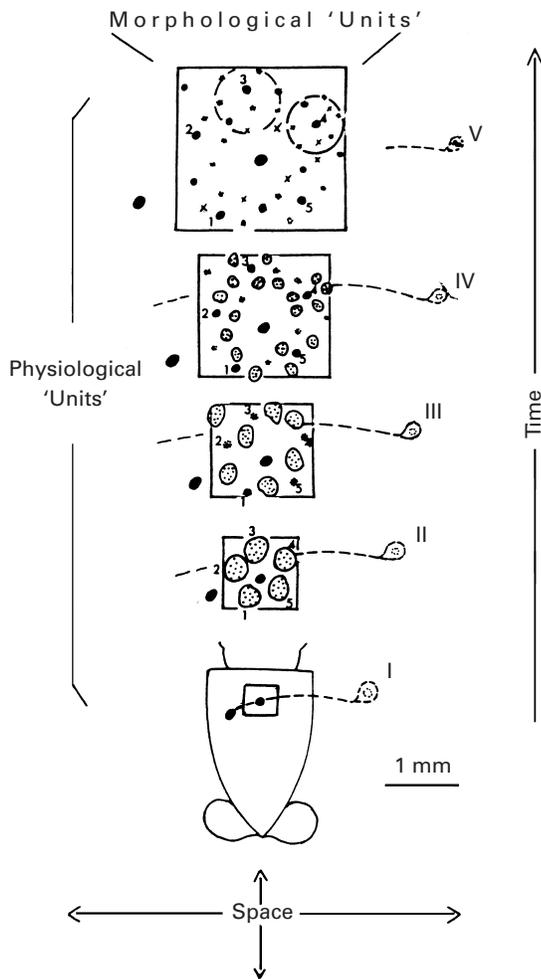


Fig. 27. Recruitment of chromatophores to form single 'morphological' units in the dorsal mantle skin of a young squid during a fivefold size increase (boxes). Five successive age classes of chromatophore (I-V) differing in size and colour are depicted, each with its own motoneuron (right). At first appearance, age-classes II-IV are shown physiologically expanded, through contraction of their radial muscles. Members of age-class V (x) are still being born and their nerve supply is in the process of growing in. In this last box we can see two new 'morphological units' being created, centred on chromatophores 3 and 4 (age class II) (Packard, 1982).

officinalis, the chromatophores are sparser over the white skin areas rich in leucophores, such as the white zebra bands or the White fin spots (Fig. 19), which are so important for signalling (Section VII). The interaction of leucophores and chromatophores in development is a subject ripe for investigation, as is the question of leucophore development.

In one cephalopod, *Sepia officinalis*, the changes in body patterning from hatching to adulthood have been followed in some detail (Hanlon & Messenger, 1988). In this species, it has been shown that some

components of body patterns drop out of the repertoire with age, while others arise later in ontogeny. The changes relate to the changing function of the body patterns over the life cycle, with an emphasis on concealment in the early stages and on signalling later on. Changes in camouflage strategy with increasing body size and decreasing chromatophore density have also been documented in this species by Hanlon & Messenger (1988): see Fig. 39 and Section VII.

Although cephalopods are characterized by their remarkable learning abilities (for a review see Hanlon & Messenger, 1996) there is no indication that body patterns are learned. In the only study that specifically addresses this problem, Warren, Scheier & Riley (1974) described the body patterns shown by *Octopus rubescens* during attacks on free-swimming crabs and on tethered crabs and compared them with those shown during training on a simple visual discrimination task. They found that there were no changes in body patterning at any stage of the learning process; moreover the changes in patterning seen during attacks on the conditioned stimulus were identical with those occurring during attacks on crabs. In short, they concluded that colour change in this species was 'tied to locomotor acts and postural adjustments'. They also noted that, during attacks on prey, octopuses often exhibited body patterns that bore no relation to the substrate, a point we shall return to below (Section VII.3).

V. THE CHROMATOPHORE SYSTEM IN THE BRAIN

The cephalopod brain is extremely well developed for an invertebrate, with the ganglia centralised and arranged in a series of discrete lobes around the gut (Budelmann, 1995). Its functional organization has been the subject of innumerable studies, notably by J. Z. Young and his colleagues. The key references are: for *Sepia officinalis*, Boycott (1961); for *Loligo* spp., Young (1974, 1976, 1977a, 1979) and Messenger (1979a); and for *Octopus vulgaris*, Young (1971).

Several brain areas influencing the chromatophores had been identified by earlier workers (Klemensiewicz, 1878; Phisalix, 1894, von Uexküll, 1895, Polimanti, 1913), but it was the direct electrical stimulation experiments of Boycott (1961) on *Sepia officinalis*, coupled with anatomical investigations based on reduced silver staining, that established unambiguously the essential organ-

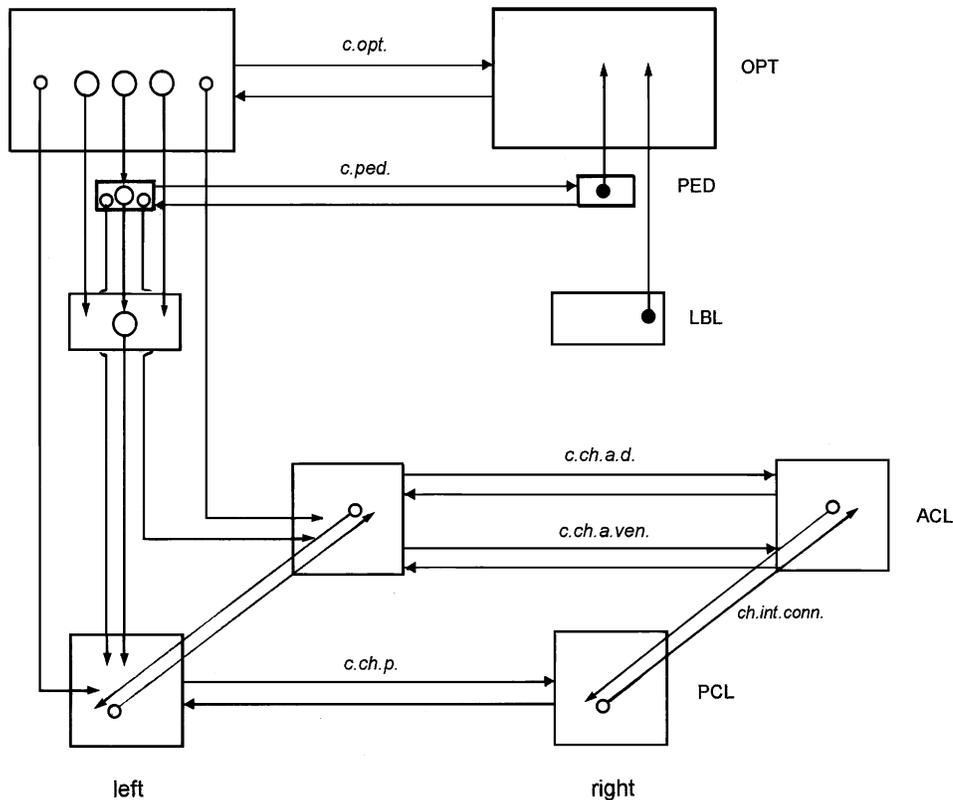


Fig. 28. Simplified scheme showing relationship of the principal brain lobes controlling body patterning in *Octopus vulgaris*. Descending pathways shown on the left, ascending ones on the right (cells shown as filled circles). Size and numbers of circles indicates relative importance of pathway. OPT, optic lobe; PED, peduncle lobe; LBL, lateral basal lobe; ACL, PCL, anterior and posterior chromatophore lobes, respectively; c.opt., optic commissure; c.ped., peduncle lobe commissure; c.ch.a.d., c.ch.a.ven., dorsal and ventral anterior chromatophore lobe commissures, respectively; c.ch.p., posterior chromatophore lobe commissure; ch.int.conn, inter-chromatophore lobe connective. Based on data from Young (1971), Camm (1986), and J. B. Messenger (unpublished observations).

Table 5. Numbers of fibres linking the four chromatophore lobes in *Octopus vulgaris*

Tract	Number of fibres
Posterior chromatophore commissure	24161
Anterior chromatophore commissure	
Ventral	24770
Dorsal	24000
Total	48700
Inter-chromatophore lobe connective	173268 (one side only)

Estimates based on counts of electron microscope sections: most fibres $< 0.5 \mu\text{m}$ in diameter, modal values around $0.3 \mu\text{m}$. Based on Camm (1986).

ization of the chromatophore control system in the brain. His findings have subsequently been extended,

and largely confirmed, by Young (1971, 1976, 1977b), Budelmann & Young (1985), Camm (1986), Dubas *et al.* (1986a, b), Saidel & Monsell (1986), and Novicki, Budelmann & Hanlon (1990), although differences between genera have emerged.

In the account that follows, we shall focus on *Octopus vulgaris*, the species that has been most studied. In Fig. 28 the organization of the chromatophore control system is shown diagrammatically: we shall see that the arrangement is a hierarchical one, with basically three levels of control. The chromatophore lobes, containing the chromatophore motoneurons, lie at the lowest level. They receive input from the lateral basal lobes, which in turn receive input from the highest level, the optic lobes. The optic lobes are, of course, processors of the visual input and in life the chromatophores are primarily, although not exclusively, visually driven. There is a second visual pathway to the lateral basal lobe, *via* the peduncle lobe.

Table 6. Cell sizes and numbers in the chromatophore lobes of *Octopus vulgaris*

Lobe	Number	Size: < 10 μm (%)	10–20 μm (%)
Anterior chromatophore lobe	108 500	79	21
Posterior chromatophore lobe	154 500	64	36
Total	263 000		
Grand total (both sides)	526 000		
Lateral basal lobe	63 500	97.5	2.5
Grand total (both sides)	127 000		

Data from Young (1963, 1971).

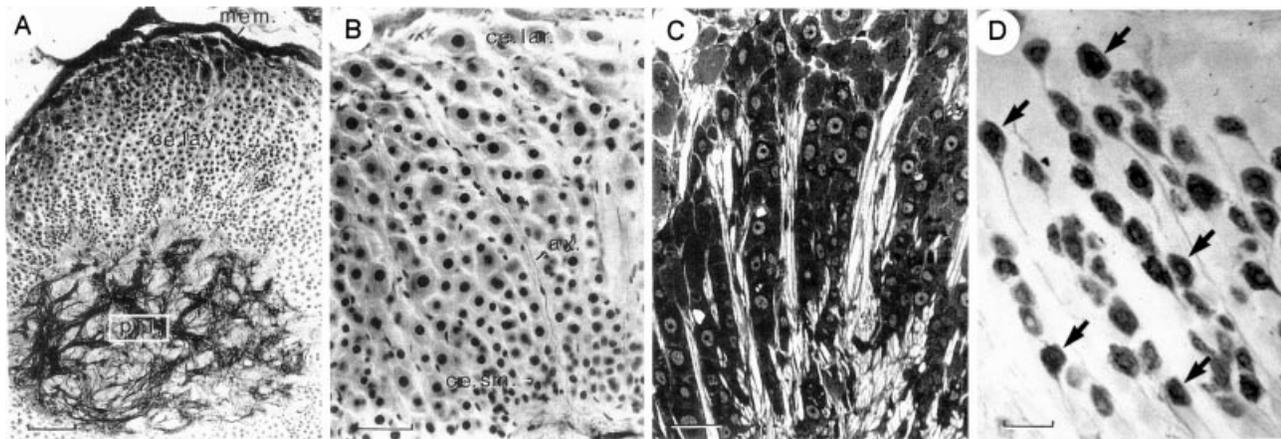


Fig. 29. The posterior chromatophore lobe (PCL) of *Octopus vulgaris* in transverse section. (A) Cajal silver stain, showing cell layer (ce.lay.) and neuropil (pil.), where a darkly staining meshwork of afferents interacts with processes from the motoneurons. mem., membrane. Scale bar, 500 μm (B) Higher power view of cell layer, showing large (ce. lar.) and small cells (ce. sm.) and a 'canal' with nerve axons (ax.). Scale bar, 60 μm . (C) Toluidine Blue stain of semi-thin section, showing columnar arrangement of cells. Scale bar, 100 μm . (D) Serotonin antiserum (and peroxidase-antiperoxidase/diaminobenzidine) stain, showing that a large proportion of PCL cell bodies contain serotonin (arrows). Scale bar, 40 μm .

(1) Anatomy

(a) The chromatophore lobes

The chromatophore motoneurons mostly lie in four lobes on the latero-dorsal aspect of the sub-oesophageal brain: the paired anterior chromatophore lobes (ACL) and the paired posterior chromatophore lobes (PCL). In *Octopus vulgaris* there are also some chromatophore motoneurons in the anterior region of the pedal lobes and in the vasomotor lobe (Budelmann & Young, 1985; Saidel & Monsell, 1986); in *Lolliguncula brevis* there are also some in the fin lobe (Dubas *et al.*, 1986*b*). In *O. vulgaris* there are two commissures linking the ACLs and a single commissure linking the PCLs; the anterior and posterior chromatophore lobes of the same side are joined together by the inter-chromatophore tract or connective (Young, 1971). Estimates

of the numbers of nerve fibres in these commissures and connectives have been made by Camm (1986), on the basis of an electron microscopic investigation. Table 5, which summarizes some of his findings, shows that there is considerable cross-talk between the four chromatophore lobes, presumably to ensure that the appearance of the skin of the entire animal is always fully coordinated. The sizes and numbers of cells in the chromatophore and lateral basal lobes are given in Table 6. Again note the high numbers: in *O. vulgaris* there are almost as many cells controlling the chromatophores as there are cells controlling the rest of the routine motor actions, such as breathing, swimming, walking and ejecting ink (Young, 1963).

As in all invertebrate brains, the cell bodies of the chromatophore lobe neurons form an outer 'rind', or cell layer, surrounding a central neuropil. This contains the trunks of the neurons, most bearing

collaterals, as well as the terminals of incoming afferent fibres. It is in the neuropil that much of the input from the other lobes of the system exerts its effect on the chromatophore motoneurons (Froesch, 1972). The chromatophore lobes are unusual, however, in that the cell layer is very thick and the cells are arranged regularly in columns, with the largest cells (over 20 μm in diameter) lying peripherally and the smallest (youngest?) next to the neuropil (Fig. 29). Furthermore there is a series of neuropilar channels extending into the cell layer up which afferent fibres run to make functional contact with chromatophore motoneurons. Most unusually, for an invertebrate, they do so *via* processes ('dendrites') on the cell body itself (Young, 1971). The significance of this remains unclear, but after lesions to the lateral basal lobe (LBL) degeneration has been found in the canals and around the 'dendrites' of the PCL, so that there is direct evidence that afferent fibres from the LBL influence chromatophore motoneurons *via* the 'dendrites' as well as *via* axon collaterals in the neuropil (Camm, 1986).

It is thought that the majority of cells in the PCL are motoneurons, but there are many cells with processes proceeding to the ACLs or to the contralateral PCL (Budermann & Young, 1985; Camm, 1986). Lucifer Yellow fills in the PCL have revealed that there are also some cells whose processes remain within the lobe, even within the cell layer: more importantly dye-fills also showed that approximately a quarter of the cells sampled (over 100 cells from nearly 50 animals) were dye-coupled (Miyan & Messenger, 1995). This agrees with ultrastructural evidence that their cell bodies are closely interlocked (Camm, 1986). Such functionally linked banks of chromatophore motoneurons could provide the neurological basis for the components of patterning described in Section VI, as Packard (1995*c*) has argued. It is not known whether coupling occurs between PCL neurons in squids.

The neuropil of the chromatophore lobes is characterized by bundles of 50–100 efferent axons running out through a loose lattice-work of afferent fibres, which stain darkly in silver preparations (Fig. 29A; Young, 1971, Froesch, 1972). Boycott (1953) has noted that the degree of regularity of the lattice-work is much higher in cephalopods that have complex body patterns (such as *Sepia officinalis* and *Octopus vulgaris*) than in those that do not (*Loligo vulgaris* and *Argonauta argo*).

Efferent fibres from the chromatophore lobes leave the brain in 20 nerves (10 on each side, Fig. 20A). The majority of ACL motoneurons innervate

chromatophores on the head and arms (but some go to the mantle) while the majority of PCL motoneurons innervate the mantle chromatophores (but some go to the arms: Budermann & Young, 1985). The chromatophore nerves probably all run directly to the chromatophore muscles in the periphery without synapsing (Sereni & Young, 1932; Dubas *et al.*, 1986*a*; Saidel & Monsell, 1986). There are apparently no efferents ascending to higher levels in the brain: the lateral basal, peduncle or optic lobes. There are, however, efferents to skin muscles controlling texture. In *Octopus vulgaris* Miyan & Messenger (1995) found that when they cut the membrane over the PCL (Fig. 29A), prior to inserting an electrode, there was erection of large papillae all over the ipsilateral mantle; and in *Sepia officinalis* Boycott (1961) observed papilla erection after stimulating in the ACL or PCL (see also Section V.5).

Afferents to the lobes derive principally from the lateral basal lobes (LBL), and severing the LBL-PCL tract produces heavy degeneration in the PCL neuropil (Froesch, 1972); but there is a very large input from the peduncle lobes and also direct pathways from the optic lobes and the statocysts (Camm, 1986). In the squid, *Lolliguncula brevis*, Novicki *et al.* (1990) found there were inputs to the ACL and PCL from the brachial, anterior pedal and posterior pedal lobes. There is no evidence to date of afferent input from the chromatophores themselves, and it needs emphasising that control of the chromatophores appears to occur without feedback (see below).

(b) *The lateral basal lobes*

These lobes lie one on each side of the posterior aspect of the supra-oesophageal brain, just above the gut. The cells of the lobe, which are for the most part smaller than those in the ACL or PCL (Table 6), lie in the posterior walls and the cell layer lacks the curious "canals" seen in the chromatophore lobes. Centrally and anteriorly their neuropils are continuous with those of the median basal and interbasal lobes, ventrally with the dorsal magnocellular lobe.

Most of the efferent fibres from the LBL run to the four chromatophore lobes, although there are projections back to the optic lobes (Young, 1971) and possibly to the peduncle lobes (J. B. Messenger, unpublished observations). In *Lolliguncula brevis* there are also efferents to the fin and magnocellular lobes (Novicki *et al.*, 1990).

The afferents derive principally from the optic lobes, although there is also a large peduncle lobe to

lateral basal tract. There is possibly a statocyst to lateral basal tract (Young, 1971); such a tract is definitely present in *Loligo* spp. (Young, 1977*b*) and probably so in *Sepia officinalis* (Ferguson, Messenger & Budelmann, 1994).

(c) *The optic lobes*

These are large, complex structures, each containing a total of approximately 65×10^6 cells in *Octopus vulgaris* (Young, 1963) and they have a variety of functions. The outer or cortical regions constitute a kind of 'deep retina' (Cajal, 1917), containing visual analysing systems to process the input from the retina itself (Young, 1971). The central medulla, with over 13×10^6 cells, is part visual memory store, part higher motor centre and may have other functions (Boycott, 1961; Young, 1971, 1974). It contains cell bodies of varying size, clumped together in characteristic 'cell islands' surrounded by neuropil; there is no obvious histological differentiation within the medulla and, despite claims by earlier workers, stimulation anywhere in this zone can lead to a whole variety of motor responses, including colour changes (see below).

Efferents from each optic lobe project widely within the brain; there is a large optic commissure and a conspicuous tract to the LBL, as well as numerous tracts to the ipsilateral peduncle lobe, which in turn projects to the LBL: the significance of this dual pathway is not clear. Curiously, in the squid, *Lolliguncula brevis*, Novicki *et al.* (1990) found that there is no direct projection from the optic lobe to the LBL: the chromatophores appear to be driven *via* the peduncle lobes. In *Octopus vulgaris* there are also tracts to the contralateral optic, peduncle and lateral basal lobes (Fig. 28) and a small projection to all four chromatophore lobes (Camm, 1986).

Afferents to the optic lobes derive from all parts of the supraoesophageal brain, including the peduncle lobe and LBL, but there is a major input from the retina, *via* approximately 20×10^6 optic nerves (Young, 1965).

(d) *The peduncle lobes*

The relatively tiny peduncle lobe lies on the optic tract in the hilum of the optic lobe; it is intimately associated with the olfactory lobe (Messenger, 1967*a*, 1971). The lobe comprises two regions: the dorsal 'spine', with uniformly small cell bodies and a neuropil containing an array of fine parallel fibres, and the basal zone, with cells of varying size and a

neuropil with a coarse meshwork of fibres staining intensely with silver. Efferents from this lobe run to many motor centres in the brain including the lateral basal lobes (Messenger, 1967*a*; Young, 1971) and the chromatophore lobes (Camm, 1986). There is also a back projection to the optic lobe, which provides the principal source of afferents to the peduncle lobe; there is a peduncle commissure and connections with the contralateral optic lobe as well as the ipsilateral one (Messenger, 1967*a*). Further details of the connectivity and function of this lobe are given in Messenger (1983) and Camm, Messenger & Tansey (1985).

(2) **Lesions to the chromatophore system**

In *Octopus vulgaris* it is relatively simple to remove the uppermost level of the system by sectioning the optic tracts. Immediately after such a lesion, which also removes the peduncle lobe (Messenger, 1967*b*), the animal lies immobile, without posture and is uniformly pale. After some days, however, such a preparation can move and feed; and chromatophore tone gradually returns. Patterning is sometimes evident, often of high contrast (e.g. 'conflict mottle': Packard & Sanders, 1969), and darkening may occur after mechanical stimulation. This is clear evidence that the chromatophore motoneurons are intact, and potentially fully functional; however, such a preparation lacks visual input so that if transferred to backgrounds differing in brightness or contrast there can be no corresponding adjustment of the chromatophores as in normal octopuses.

If the optic tracts are sectioned distal to the peduncle lobes, however, there is no pallor or general loss of muscular tone; if the peduncle lobes alone are removed, bilaterally, leaving the optic lobes intact, there appears to be little effect on the chromatophores (Messenger, 1967*b*), although such lesioned animals were not tested carefully on different backgrounds. An asymmetric lesion that removes both optic lobes but only one peduncle lobe produces a preparation in which all the chromatophores ipsilateral to the intact peduncle lobe are fully expanded (Messenger, 1967*b*). Such lesions clearly implicate the peduncle lobe in the control of the chromatophore motor neurons, but they suggest that its role is a subtle, regulatory one, as it is of other motor activities (Messenger, 1983).

It is technically difficult to remove the LBL completely but partial lesions have surprisingly little effect on the appearance of an octopus, even shortly after the operation. The effect can best be described

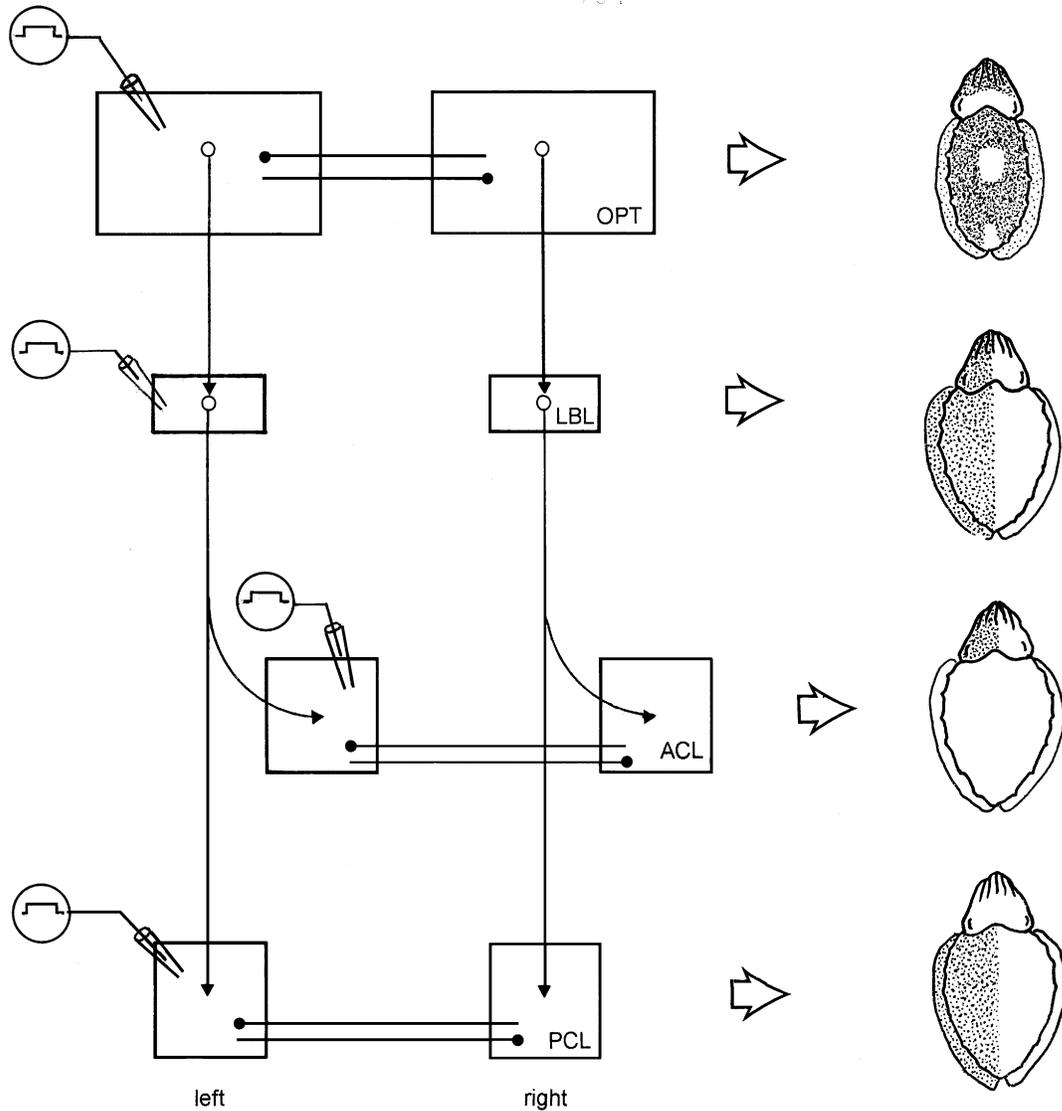


Fig. 30. Direct electrical stimulation in the lower levels of the chromatophore system in the brain of *Sepia officinalis* causes gross darkening only. Only by stimulating in the optic lobe (OPT) can a body pattern (e.g. Disruptive) be elicited. LBL, lateral basal lobe; ACL, PCL, anterior and posterior chromatophore lobes, respectively. Based on the findings of Boycott (1961).

as a 'coarsening' or an 'exaggeration' of what would otherwise be a subtle pattern. Perhaps one of the functions of this lobe is to fine-tune patterns by decreasing their contrast. Immediately after sectioning the LBL-PCL tract there is significant ipsilateral paling of the entire body (see Fig. 7 in Froesch, 1972).

It is also possible to remove the optic lobes together with the LBLs bilaterally, a lesion producing the so-called 'sub-oesophageal preparation' (Andrews *et al.*, 1983) and Packard (1995*c*) has studied such animals in detail. He found that they show little muscle tone, and appeared grey: yet the

chromatophores sometimes showed spontaneous activity, waves of colour running in different directions across the mantle, and they could be activated by vigorous stimulation. More interestingly he noted the spontaneous appearance of several normal components of body patterns, such as 'yellow screen', 'all dark', 'orange surrounds' and, in one individual, 'dorsal trellis' (Packard, 1995*c*). What these components have in common is that they provide the inconspicuous 'ground colours' of patterns, against which bold bars and spots would normally be superimposed. However, these, and other components such as skin papillae, were always

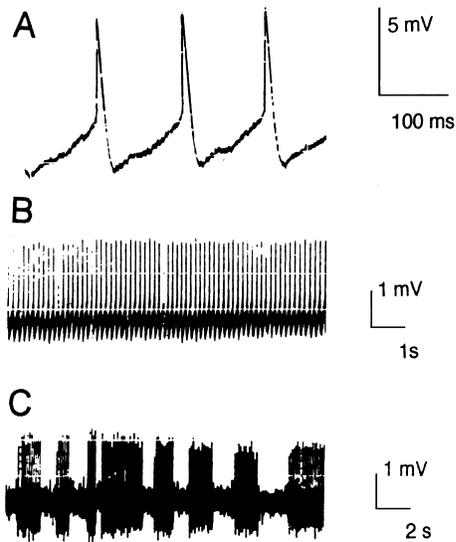


Fig. 31. Intracellular recordings from cells in the posterior chromatophore lobe (PCL) of *Octopus vulgaris*. (A) Typical action potentials on a fast time base. (B) Pattern recorded from a commonly encountered tonically firing cell, (C) Pattern recorded from a rarely encountered bursting cell (Miyayama & Messenger, 1995).

shown in isolation, and the sub-oesophageal animals never switched from component to component or modified the degree of expression of a component (see Section V.5).

Lesions to the chromatophore lobes themselves have not proved to be feasible in *Octopus vulgaris*: the ACLs are completely inaccessible for surgery using present techniques and although the PCLs are potentially approachable, exposing them interferes with the blood supply and invariably leads to death.

It has not proved possible to make lesions to the brain of *Sepia officinalis* or of loliginid squids.

(3) Stimulating and recording from the chromatophore system

Direct electrical stimulation of the lobes in the cephalopod brain influencing the chromatophores has been carried out by a number of workers but by far the most significant study is that of Boycott (1961) on *Sepia officinalis*, using hand-held electrodes in lightly anaesthetised, acute preparations. By stimulating the PCL he was able to elicit total darkening and erection of skin papillae on the mantle, generally ipsilaterally (Fig. 30); stimulating the ACL led to total darkening and erection of papillae on the head and arms (ipsilaterally). Stimulation of the LBL led to uniform darkening, either ipsilaterally or bilaterally, never paling; there was also erection of skin papillae. The responses were on the head, arms and mantle (Fig. 30). Stimulation in the optic lobe led to one of three kinds of response: darkening, paling or *patterning*, unilaterally or bilaterally. The patterns obtained included Light Mottle, Zebra and Disruptive (Fig. 30). Later, Chichery & Chanelet (1976) chronically implanted electrodes in the optic lobes of unrestrained cuttlefish and succeeded in eliciting the Deimatic pattern by direct electrical stimulation. When electrodes were implanted in the peduncle lobes, however, stimulation produced only darkening (Chichery & Chanelet, 1978).

The implications from these experiments are clear and agree with the connectivity within the hierarchical system outlined above. Chromatophore motoneurons in the ACL and PCL run principally to the ipsilateral anterior and posterior regions of the body, respectively; crude electrical stimulation here will cause gross but spatially limited chromatophore

Table 7. *Distribution of putative transmitters in the chromatophore system in the cephalopod brain*

Lobe	ACh*	DA/NA	OA	5-HT	L-glu	GABA	NO	Peptides
ACL/PCL	pil	pil	?	cells/pil	cells	pil	(pil)	cells
LBL	?	cells/pil	?	cells/pil	?	pil	pil	?
Optic lobe	cells/pil	cells/pil	✓	cells/pil	?	cells/pil	(cells)	?

ACL, PCL, anterior and posterior chromatophore lobe respectively; LBL, lateral basal lobe; pil, neuropil; ACh, acetylcholine, *strictly acetylcholinesterase; DA, dopamine; NA, noradrenaline; OA, octopamine; 5-HT, serotonin; L-glu, L-glutamate; GABA, γ -amino butyric acid; NO, nitric oxide; (), weak response; ?, no evidence at present; ✓, present in homogenate.

Data from *Octopus vulgaris* (columns 2–7) and *Sepia officinalis* (columns 8 & 9), based on Andrews *et al.* (1981, 1983), Di Cosmo *et al.* (2000), Cornwell *et al.* (1993), Juorio (1971), Juorio & Molinoff (1974), Kime & Messenger (1990), Loi & Tublitz (2000), J. B. Messenger, unpublished data, Parr (1988), Tansey (1980), Uemura *et al.* (1987).

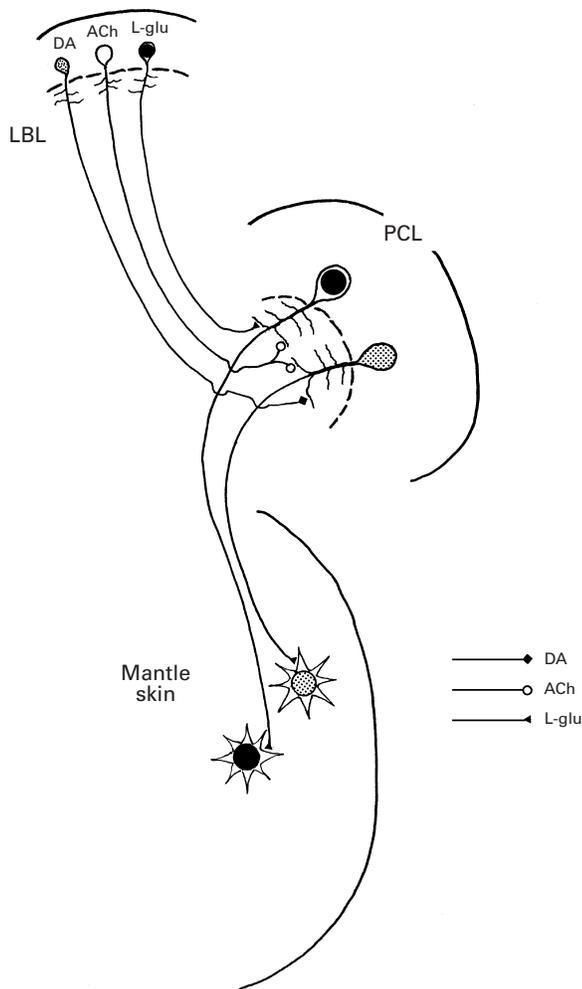


Fig. 32. Multiplicity of neurotransmitters in the chromatophore system in the brain of *Octopus vulgaris*. There are at least two classes of motoneuron in the posterior chromatophore lobe (PCL). One, excited by dopamine (DA, \blacklozenge), expands yellow, orange and red chromatophores (stippled); the other, excited by L-glutamate (L-glu, \blacktriangle), excites black chromatophores. Both are inhibited by acetylcholine (ACh, \circ). This suggests that the lateral basal lobe (LBL) contains at least three pharmacologically distinct types of cell (see text).

responses. The LBL projects to the ACL and the PCL, so stimulating here produces the darkening of the entire body, unilaterally or bilaterally (for there are cross projections as well as commissures). But inhibition of motoneurons and selective excitation-plus-inhibition of populations of chromatophore motoneurons to produce patterning are properties of cells in the optic lobe.

There are comparable, although less complete data for *Octopus vulgaris*. Stimulation of the peduncle

lobe elicits chromatophore responses, as was shown long ago by Klemensiewicz (1878), von Uexküll (1895) and Polimanti (1913) but these are limited to gross darkening (Messenger, 1967*a*) and although Young (1971) reports patterning as a result of stimulating at the centre of the optic lobe it was never normal.

Recording from the brain of cephalopods has proved notoriously difficult, but some data are available from the chromatophore system. Mislin, Riesterer & Schroeder (1954) recorded extracellularly in the PCL of *Octopus*, noting particularly many 'spontaneously' active cells firing at frequencies of between 10 and 60 Hz. He also found that lower temperatures could induce bursts of firing from PCL neurons, a finding particularly interesting in the light of the experiments of Andrews, Packard & Tansey (1982) (see Section V.5). More recently Miyan & Messenger (1995), who developed a preparation that allowed intracellular penetration of cells in the PCL in a restrained, cooled octopus, succeeded in penetrating over a hundred PCL neurons, stimulating and recording from them. Stimulation produced small chromatomotor fields scattered across the mantle: there was no evidence of somatotopy. Recording showed that most of the cells were spontaneously active, firing at from 1 to 12 Hz: such cells presumably maintain the tonic activity needed in a living octopus to sustain camouflage against its predators. There were also a few 'bursting' cells (Fig. 31) whose function is unknown.

In the squid, *Lolliguncula brevis*, Dubas *et al.* (1986*a*), using a perfused, semi-intact preparation, stimulated motoneurons extracellularly in the PCL and also stimulated the pallial nerve, using a cuff electrode. Like Boycott (1961), they obtained only expansion of the chromatophores, confirming Florey's (1969) claim that there are no inhibitory chromatophore motoneurons; unlike him, however, they obtained local rather than gross effects. They found compact chromatomotor units that generally comprised 6–10 chromatophores, and although there were some on the fins with as many as 60 chromatophores the great majority contained less than 20. Perhaps the most striking finding of Dubas *et al.* (1986*a*) was that adjacent motoneurons in the PCL do not necessarily have adjacent chromatomotor fields on the mantle. They also found that the latency of the chromatophore response was constant at different train frequencies, whether the PCL or the pallial nerve was stimulated, in keeping with the evidence that the chromatophores are controlled *via* monosynaptic pathways.



Fig. 34. Visual feedback cannot be involved in the regulation of body patterns: a plastic ruff placed around the 'neck' of *Sepia officinalis* (mantle length 120 mm) prevents it from seeing its own mantle but does not prevent it showing an appropriate Disruptive pattern, which includes such distinctive components as the White square.

(4) The neurotransmitters of the chromatophore system

It should be apparent by now that the system in the brain controlling the chromatophores, especially in such inshore genera as *Sepia* and *Octopus*, is complex, comprising millions of neurons. An added complexity is its pharmacology: all the 'classic' neurotransmitters – acetylcholine (ACh), dopamine (DA), noradrenaline (NA), serotonin (5-HT), γ -aminobutyric acid (GABA) and L-glutamate (L-glu) – are present in some parts of the system, as well as octopamine (OA) and, in some cephalopods the FMRamide-related family of peptides (FaRPs). There are even a few fibres in the PCL that stain positively for nitric oxide synthase (Di Cosmo *et al.*, 2000) (Table 7).

The evidence for this comes from several different kinds of investigation: chemical identification of specific brain regions after dissection (Juorio, 1971; Juorio & Molinoff, 1974; Kime & Messenger, 1990); cholinesterase- and fluorescence-histochemistry (Barlow, 1977; Tansey, 1980; A. Di Cosmo, & J. B. Messenger, in preparation); immunohistochemistry (Uemura *et al.*, 1987; Parr, 1988; Cornwell, Messenger & Williamson, 1993; Cornwell & Messenger, 1995; Messenger, Cornwell & Reed, 1997; Loi *et al.*, 1996); and physiological experiments (Sereni, 1930; Chichery & Chanelet, 1972; Andrews *et al.*, 1981, 1983; J. B. Messenger, unpublished observations). For a detailed review, see Messenger (1996).

The distribution of the various transmitters in the chromatophore system is listed in Table 7, and it is

appropriate to summarize the principal findings for *Octopus vulgaris*. (1) acetyl cholinesterase is present in the neuropil of all the chromatophore lobes and the optic lobes and possibly in the lateral basal lobes (Tansey, 1979); and the high levels of ACh in the optic lobe (see references in Messenger, 1996) suggest a major role for ACh in chromatophore control; (2) biogenic amines are widely distributed in the system: there are especially high levels of dopamine (DA) and noradrenaline (NA), and these are present in the neuropils of the LBL and PCL (Tansey, 1979); (3) 5-HT is also widespread (Kime & Messenger, 1990) and has been immunohistologically – localised in a large proportion of cell bodies in the ACL and PCL (Uemura *et al.*, 1987; Parr, 1988; Fig. 29D); (4) the majority of cell bodies in the PCL stain positively with antibodies to L-glutamate (J. B. Messenger, unpublished data) and there are comparable findings for *Sepia officinalis* (Loi & Tublitz, 2000).

Some of these data are in agreement with the pharmacological findings discussed in Section II: in particular, we may note the presence, centrally in *Octopus vulgaris*, of two substances active peripherally in loliginid squids: L-glu and 5-HT. Furthermore, the data take on more significance in the light of the experiments of Andrews *et al.* (1981, 1983). These involved direct perfusion *in vivo* of small (100 μ l) 'pulses' of transmitters (their agonists or antagonists) at physiological dose levels through a cannula implanted in the cephalic aorta of an octopus immediately posterior to the brain. Such injections produced immediate although transient effects on the motor system, including dramatic effects on the chromatophores, as Sereni (1930) had found earlier. The chromatic responses can be summarised as darkening, paling or patterning.

Darkening in *Octopus vulgaris* can involve 'orange' chromatophores (strictly, any of the yellow-orange-red series, Section II) or black ones. The former expand to darken the animal when catecholamines or their α -agonists, or octopamine is injected. If L-glu (or its agonists, kainate or quisqualate) is injected, only the black chromatophores expand. This suggests that the red and black chromatophores not only constitute separate populations, but that their respective motoneurons in the PCL are activated by different transmitter substances (Fig. 32). Paling of the red chromatophores can be brought about by perfusing substances such as phentolamine, which are α -blockers. Complete whitening, however, occurs after perfusing the preparation with ACh (or nicotine): that is, both black and red chromatophore

motoneurons in the PCL receive an inhibitory cholinergic innervation centrally. Since similar results to these were obtained after brain lesions that removed the optic and lateral basal lobes, Andrews *et al.* (1983) concluded that the receptors involved are in the suboesophageal lobes, by implication on neurons in the ACLs and PCLs themselves, although this has yet to be confirmed.

One of the most striking results obtained in the perfusion experiments with *Octopus vulgaris* was that perfusion with 5-HT produces a striking mottled pattern (Fig. 33, Plate 2). This is remarkable evidence that the perfused substances in these experiments are acting on specific neurons in the brain. Moreover the pattern elicited by 5-HT is almost identical to that shown by living octopuses in a conflict situation (Packard & Sanders, 1969). How 5-HT acts to produce this pattern remains a matter for speculation, but, since it is possible to elicit it after the upper levels of the brain have been surgically removed, it is clear that the 5-HT receptors involved must lie at the lowest levels of the system, i.e. in the PCL (Andrews *et al.*, 1983).

The pharmacological data are obviously incomplete and sketchy, but they are consistent and suggest that, in *Octopus vulgaris*, separate pathways have evolved in the chromatophore system that use their own 'private' transmitters. Thus the motoneurons driving the black chromatophores are excited by L-glu, the motoneurons driving the red chromatophores are excited by NA and/or DA (Fig. 32). Both classes of motoneuron are inhibited by ACh, whose effect in the central nervous system of cephalopods is generally inhibitory (Messenger, 1996).

Support for the presence of different endogenous transmitters in the chromatophore control system comes from the degeneration study of Froesch (1972), who found at least two distinctive populations (perhaps more) of synaptic vesicles in the PCL associated with the LBL input: clear 37 nm diameter vesicles, and dense 84 nm diameter vesicles.

(5) A tentative synthesis

The extraordinary complexity of the chromatophores peripherally (Sections II and III) is clearly matched by complexity in the brain, especially in such species as *Octopus vulgaris* and *Sepia officinalis*. The chromatophores are controlled by no fewer than five pairs of lobes in the brain, organized hierarchically (Fig. 28). In *O. vulgaris* there are over

600 000 cells in the lower parts of the system (the chromatophore and lateral basal lobes) and the details of their connectivity still elude us. The chromatophore motoneurons are nearly all situated in the ACLs and PCLs, which are linked by numerous connective and commissural fibres (Table 5). In *O. vulgaris* many motoneurons in the PCL are dye-coupled and are tonically active. In life, their firing is presumably enhanced or inhibited, as appropriate, by descending pathways from the LBL and also from the optic and peduncle lobes, as lesion experiments reveal. After removal of the optic, peduncle and lateral basal lobes, or section of the LBL-PCL tracts, patterning is initially abolished, although some components can reappear. Gross electrical stimulation of the ACL or PCL causes total expansion of the chromatophores on the head and arms or the mantle, respectively. Focal stimulation of motoneurons in the PCL of a squid, *Lolliguncula brevis*, leads to the expansion of compact chromatophore fields comprising 2–20 chromatophores. There are complementary data for *O. vulgaris*.

Taken together, these findings suggest that the chromatophore lobes contain all the motor machinery for generating the components of body patterns, but that they require appropriate instructions from higher levels in the brain if meaningful and subtle, modulated patterns are to appear. Afferents to the chromatophore lobes derive mainly from the LBLs, but the function of these lobes is far from clear. Gross stimulation here leads to total darkening of the anterior and posterior regions of the body, but never paling or patterning. Yet, in the absence of the optic lobes, they can apparently generate patterns comprising several chromatic (and textural) components (Packard, 1995*c*), although such patterns are 'stiff, exaggerated ... and seem to lack the fine details ... of normal patterns'. Those fine details, visible for example in Fig. 34 (see also Figs. 36 (Plate 3), 39 and 42), are caused by what Packard (1995*c*) terms 'spatially graded chromatophore activity', involving the actual numbers of chromatophores active, the extent of expansion of individual chromatophores and numbers of motor units and complex interactions between them. These 'amplitude effects', which may be voltage and/or frequency dependent (Dubas & Boyle, 1985; Ferguson *et al.*, 1988), perhaps depend on the LBLs, which are clearly more than simple relay stations. The exact way in which they modulate or 'refine' patterns in the intact animal remains obscure, however.

The optic lobes represent the top level of the

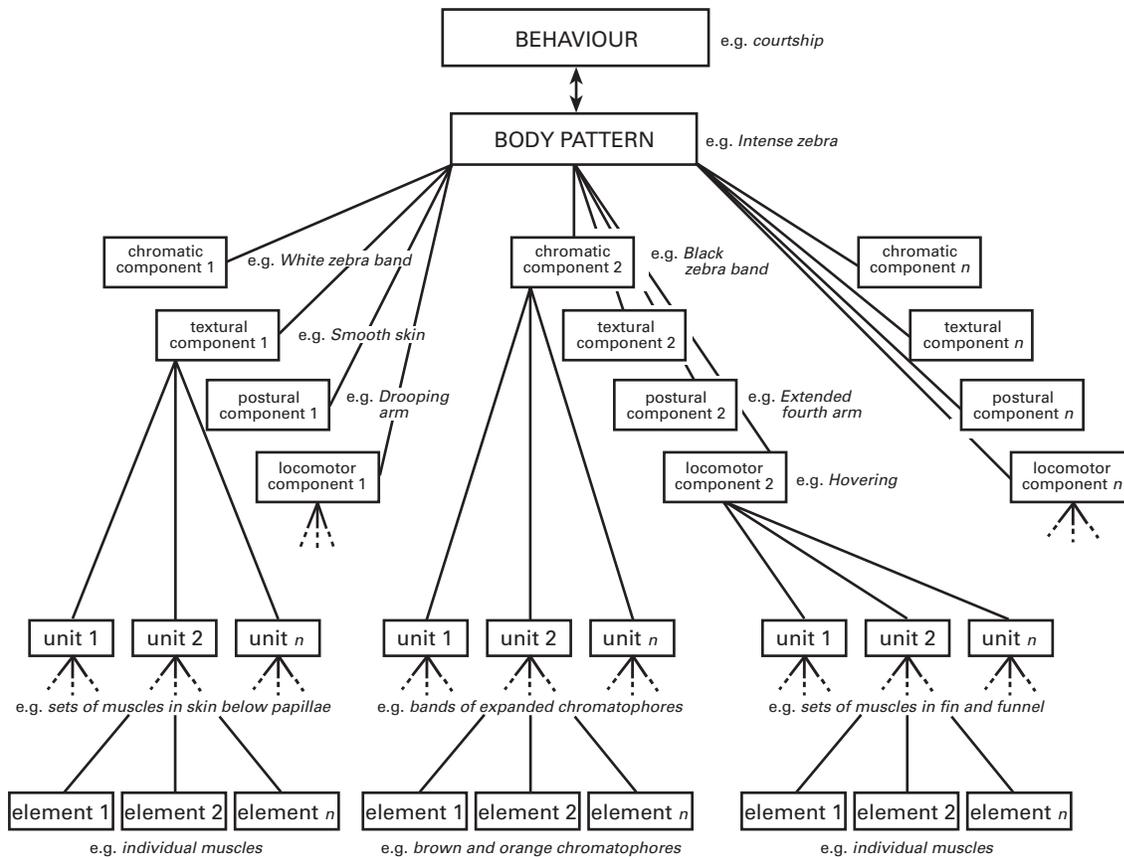


Fig. 35. Hierarchical classification of body patterning, as it applies to the Intense Zebra body pattern of an adult male *Sepia officinalis*: see Fig. 36G (modified from Hanlon & Messenger, 1988).

chromatophore system in the brain, and in life the chromatophores are largely driven by visual information (but see Section VII). The optic lobes project to all the lower lobes of the system but principally to the LBLs, and it is surely significant that, in living *Sepia officinalis*, electrical stimulation in the brain only elicits normal body patterns when applied to the optic lobes. However, the cells responsible for patterning lie among several million cells in the optic lobe medulla, and there is no evidence for any localisation of function within this region. It is generally assumed that the optic lobes are ultimately responsible for body patterning. They contain sets of cells responsible for many other specific motor programs, including programs for swimming, attacking prey, and escape-jetting (Boycott, 1961; Young, 1971); the 'body pattern cells' somehow drive chromatophore motoneurons in the ACLs and PCLs, *via* the LBLs, to produce the appropriate body pattern. However, Packard (1995*b*) claims that their role is limited to selection and modulation only, the pattern generators residing in the LBLs.

Whatever the truth it seems clear that in those

species that have been closely studied (Packard & Sanders, 1969; Hanlon & Messenger, 1988) there is not an infinite number of body patterns, but only a dozen or more (although these can be expressed in a graded way: see Section VI). In other words, there may be only about a dozen chromatophore motor programs 'hard-wired' in the brain. In *Sepia officinalis*, at least, these are nearly all fully functional at hatching (Hanlon & Messenger, 1988).

This patently sketchy account of the central control and regulation of the chromatophores reflects the unsatisfactory state of our knowledge of this system at present, and there is still much room for speculation. For example Packard (1995*b, c*) assigns particular functions to the various lobes regulating the chromatophores, such as 'gain control' for the optic lobes and 'integration' for the LBLs. However, we urgently await new technical advances to further our understanding of this whole process.

Paradoxically one apparent complication of the system – the presence of all the classical neurotransmitters – may turn out to help investigators rather than hinder them. In *Octopus vulgaris* experiments have suggested that there are receptors for many

transmitters in the chromatophore lobes themselves. Thus motoneurons driving black chromatophores are excited by perfused L-glu (or its agonists) and motoneurons driving orange chromatophores are excited by perfused NA and DA (or their agonists). Both classes of motoneuron are inhibited by descending cholinergic pathways. The use of so many transmitters in the chromatophore control system seems extravagant, but it may be necessary to 'label' and maintain separate the lines innervating particular colour classes of chromatophore. Whether or not this is true, the multiplicity of neurotransmitters does offer neurobiologists the chance of identifying, or dissecting out, some of the pathways within this multi-channel system.

Other ways of identifying specific populations of chromatophores include treatment by different anaesthetics and subject animals to severe cold. Octopuses cooled to around 4 °C display flashing brown spots all over the body: these spots normally act as screens for the centres of patches in the white spot areas (Section VI), and their motoneurons are evidently temperature sensitive (see Section V.3; Andrews *et al.*, 1982).

A final point of note: cephalopod chromatophores appear to be controlled entirely in an 'open-loop' manner. There is no evidence for any kind of mechanoreceptor associated with the chromatophores; certainly there are none in the radial muscles. Young (1971) makes the point that the

flickering of the chromatophores often seen in life may be a consequence of this lack of feedback. Moreover, it seems highly unlikely that the animals use visual feedback to regulate the body patterns. If a ruff is placed around the 'neck' of a cuttlefish, shielding the mantle from the eyes, the animal still produces (and maintains) the appropriate pattern over the whole body, even though it cannot see many of the components it is producing in its skin (Fig. 34).

VI. ASSEMBLING BODY PATTERNS

Although we are still some way from understanding the central control of body patterning in cephalopods the careful analysis of body patterns in the living whole animal, initiated by Packard and his collaborators (Packard & Sanders, 1971; Packard & Hochberg, 1977) in *Octopus vulgaris* has provided a very useful conceptual framework for understanding how patterns are assembled for the viewer. For we should always remember that the body patterns of cephalopods have evolved to confound eyes: the eyes of visual predators.

According to Packard's scheme a body pattern is built out of several components, each composed of many units: these, in turn, each contain combinations of elements, the chromatophores themselves and also reflecting cells and skin muscles (Fig. 35). It

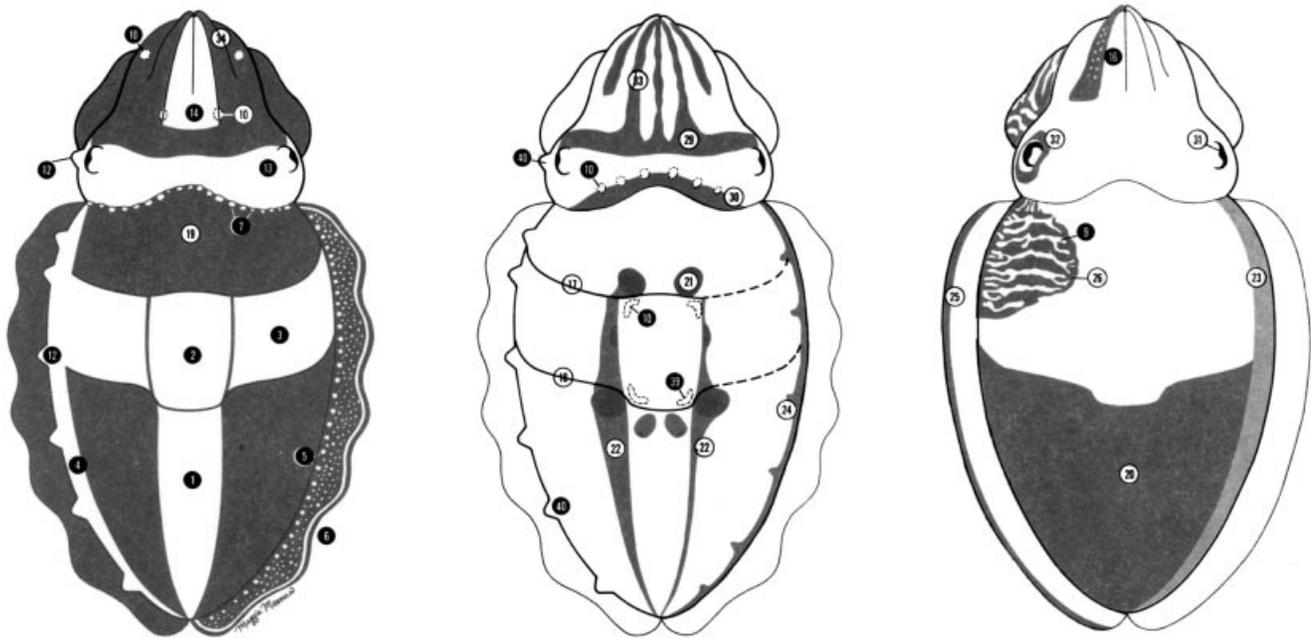


Fig. 37. Diagrammatic representation of some of the 34 (numbered) chromatic components of body patterning in *Sepia officinalis*. (Components 39 and 40 are textural components) (Hanlon & Messenger, 1988).

is convenient to consider this hierarchy in reverse order, beginning with the ‘elements’.

(1) Chromatophores as ‘elements’ in Packard’s hierarchy

Chromatophores are the structures that produce the colours yellow, orange, red and brown in the skin; in *Octopus vulgaris* there are also black chromatophores. The colour produced by an individual chromatophore will depend on how thinly its pigment granules are spread out (as the radial muscles contract more and more). The colour of a population of chromatophores (i.e., on a patch of skin) will further depend, among other things, on the proportion of the differently coloured chromatophores that are expanded; on the quality of light striking them; and on whether some of that light comes from below (after reflection from underlying structures such as muscle or leucophores). Expansion of the black or red chromatophores, especially in the sea, where the transmission of longer (and shorter) wavelengths is attenuated (Tyler & Smith, 1970), will darken the animal and help match its brightness to that of the background. These chromatophores are effectively functioning as ‘neutral density filters’ (Packard & Hochberg, 1977); and, where they overlay white areas they can ‘produce’ whites when they fully retract, because now light can reach underlying structures in the skin, notably leucophores (see Figs 34, 36, 42).

Most loliginid squids lack leucophores and complete retraction of the chromatophores produces instead the transparency that can be so important for camouflage in epipelagic animals (Mäthger & Denton, 2001).

(2) Other elements: reflecting cells and muscles

(a) Iridophores

These are multi-layer stacks of thin chitin platelets alternating with layers of cytoplasm; the platelets function as ‘ideal’ quarter-wavelength reflectors and produce spectral colours by constructive interference although they are themselves colourless (for a discussion of the physics of these cells see Denton & Land, 1971; Land, 1972). They produce the blues and greens seen in many species of cephalopod: for example, in the blue eye-spot ring of *Octopus bimaculatus* (Fig. 36A); in the greens of the belly of *Sepia officinalis* (Fig. 36G) and in the blue-greens seen

in the centre of the patch units of *Octopus vulgaris* (Fig. 16). Iridophores are also widely distributed in the skin of loliginid squids (Mirow, 1972*b*; Mäthger & Denton, 2001).

(b) Reflector cells

These have only been described in *Octopus dofleini* (Brocco & Cloney, 1980); they are complex, bearing peripheral sets of leaf-like lamellae, termed reflectosomes, approximately 1.7 μm in diameter and containing stacks of proteinaceous platelets 90 nm thick, spaced 60 nm apart. They lie beneath the chromatophore organs and are thought to function as thin-film interference devices, responsible for the blue-green reflections seen in the living animal.

(c) Leucophores

These are elongated, flattened cells, approximately 20 μm long, covered with over 1000 tiny, stalked ‘knobs’, the leucosomes (Fig. 15). They are colourless but refractile, the leucosomes scattering light to produce the chalky whites seen in incident white light. They occur in high concentrations in the various ‘white spots’ in several species of *Octopus* that are so crucial in camouflage for disruptive or ‘epistreptic’ effects (Cott, 1940; Packard, 1988*b*; see Fig. 36F). In *Sepia officinalis* they form the white spots and bands used for signalling (Hanlon & Messenger, 1988: Fig. 19B, 36G) and they are probably responsible for the white spots of *Sepioteuthis sepioidea*.

It should be recalled, however, that the leucophores will faithfully reflect incident light across the entire visible spectrum and that the so-called ‘white’ areas will appear blue in blue light or red in red light (Messenger, 1974). This may enable the skin to match the hue of the background as well as its brightness (Section VII.1*a*, Fig. 38).

(d) Skin muscles

In cuttlefishes and many species of octopods there are muscles in the skin organized to form papillae that can be major textural components of body patterns. The papillae can be mere bumps on the skin or they can be extended as spikes more than 10 mm high, such as those over the eyes, which transform the appearance of the animal (Packard & Sanders, 1969, 1971). Newly hatched *Sepia officinalis* show spikes when they are surrounded by rough coralline algae (Fig. 36H), and do so even when in a glass vessel, showing that visual cues alone are used

to generate the rough textures that aid camouflage in such an environment (Hanlon & Messenger, 1988). In *Octopus vulgaris* the papillae are centred over the circular patches (see below), so that they elevate the leucophores above their surroundings, maximizing the efficiency of these broad-band reflectors (Section VII.1a). Many octopuses can erect conspicuous dark papillae over the eyes; these may function as signals (Section VII.2).

(e) *Body muscles*

The muscles of the arms, head and mantle also contribute ultimately to the body pattern, for the various qualities of light and texture produced by the different 'elements' in the skin will be displayed on regions of the body, e.g. the arms, whose attitude can be varied. The animal may, in addition, be moving in a particular way as the elements are exhibited so that posture and locomotion contribute to the final appearance as well as colour and texture (Fig. 35, 36H).

(3) **Units**

The elements that we have described are not present in all parts of the skin of a cephalopod, nor are they uniformly distributed where they do occur: instead they appear to be organized into 'units'. At least this is certainly true of *Octopus vulgaris*, where units were first described (Packard & Hochberg, 1977). In this species, the whole body is covered by circular patches, approximately 1 mm in diameter, surrounded by a groove (Figure 36B). Distributed within each patch are chromatophores, iridophores and leucophores but their arrangement is not random. The chromatophores occur across the whole patch, as do the iridophores; the leucophores occur only in the central region of the patch (Fig. 16). Exactly at the centre, although not necessarily expressed, is the papilla; the musculature of the skin is such that the groove surrounding the patch may be deep or shallow and the patch may be smooth or rough. Ultimately, if the papilla is erected, most of the 'patch' becomes a 'spike'.

Moreover, we should recall that the chromatophores themselves are also distributed in a non-random way: the yellow-red ones of *Octopus vulgaris* are interspersed among the black ones (Section IV). The effect of this is to create two screens of different optical density for half-tone matching of the background.

In other cephalopods the 'units' are not always so obvious. In loliginid squids the 'standard discoid

unit' (Hanlon, 1982) seems to occur in all species but in *Sepia officinalis* there are no obvious units (Hanlon & Messenger, 1988).

(4) **Components**

The most conspicuous 'building blocks' of body patterns are the chromatic components; these are easily defined and occur in the same region of the body. They can be 'light' (when dark chromatophores are retracted and reflecting elements are revealed) or 'dark' (when dark chromatophores are expanded and reflection is minimal). The numbers of chromatic components in various cephalopods varies from 11 to 35 (Hanlon & Messenger, 1996) and some examples from the three major orders of cephalopods are given here. However, it is essential to note that there are also textural, postural and locomotor components contributing to body patterning.

For example, in *Sepia officinalis* 34 chromatic components can be recognised (Fig. 37), but since there are also six textural, eight postural and seven locomotor components the animal has more than 50 components available to it for generating a body pattern (Hanlon & Messenger, 1988). The small sepiid, *Metasepia pfefferi*, has 17 chromatic, 14 textural, 11 postural and seven locomotor components (Roper & Hochberg, 1988).

Among loliginid squids *Loligo forbesi* has 17 chromatic, nine postural and six locomotor components (Porteiro, Martins & Hanlon, 1990); *L. vulgaris reynaudi* had 23 chromatic, four postural and nine locomotor components (Hanlon, Smale & Sauer, 1994); *L. pealei* has 34 chromatic, five postural and 12 locomotor components (Hanlon *et al.*, 1999b); and *Sepioteuthis sepioidea* has at least 23 chromatic components (Moynihan & Rodaniche, 1982). It is worth noting that in several loliginids one of the light chromatic components can be the gonad (Boycott, 1965; Hanlon *et al.* 1994; Boal & Gonzalez, 1998) but this is revealed (or concealed) by the activity of overlying chromatophores.

Among octopods *Octopus vulgaris* has 19 chromatic components, six textural, 14 postural and four locomotor components (Packard & Sanders, 1971); *O. burryi* has 12 chromatic, four textural, eight postural and two locomotor components (Hanlon & Hixon, 1980); *O. bimaculoides* has 18 chromatic, five textural, 11 postural and two locomotor components (Forsythe & Hanlon, 1988); and *O. briareus* has 18 chromatic, four textural, nine postural and four

locomotor components (Hanlon & Wolterding, 1989).

These numbers give some idea of the numbers of chromatic components available to some common inshore cephalopods, and also the great variety of other types of component contributing to the final appearance of a cephalopod, its body pattern.

(5) Body patterns

Selected members of each of the types of component combine together to produce the final appearance of the individual cephalopod, its body pattern. Components of different or the same categories appear simultaneously (in parallel) but they may be expressed with different intensities, ranging from the barely visible to the most intense. This gradation has given rise, not surprisingly, to the mistaken belief that cephalopods can express an infinite number of body patterns. On the contrary, careful studies of particular species have revealed that all of them have only a few basic body patterns.

In *Alloteuthis subulata*, Cornwell *et al.*, (1997) recognise only three; in *Lolliguncula brevis* Dubas *et al.* (1986*a*) list four or five; in *Loligo pealei* Hanlon *et al.* (1999*b*) list 11; in *Octopus vulgaris* Packard & Sanders (1971) list 12; and even in *Sepia officinalis*, with its remarkably complex body patterning, Hanlon & Messenger (1988) recognise only 13 body patterns.

Body patterns may be chronic or acute. The former, which may be worn for hours at a time, include the patterns shown by animals such as *Octopus vulgaris* or *Sepia officinalis* on the seabed, and the countershading patterns of squids cruising in the water column. Thus their function is primarily for camouflage and they are used extensively for 'primary' defence (Edmunds, 1974; Hanlon & Messenger, 1996). Acute patterns are shown for seconds or minutes, usually by animals that are interacting with conspecifics or predators; that is, they are involved in secondary defence, brought into play once the cephalopod has been detected by a predator, or in signalling (Hanlon & Messenger, 1996). Chronic body patterns are rather variable, because natural substrates vary considerably. Acute patterns, which include deimatic or warning displays, tend to be stereotyped and, furthermore, appear to be highly conserved (Section VII.2).

The number and complexity of body patterns varies considerably among cephalopods, and even within a genus there are considerable species

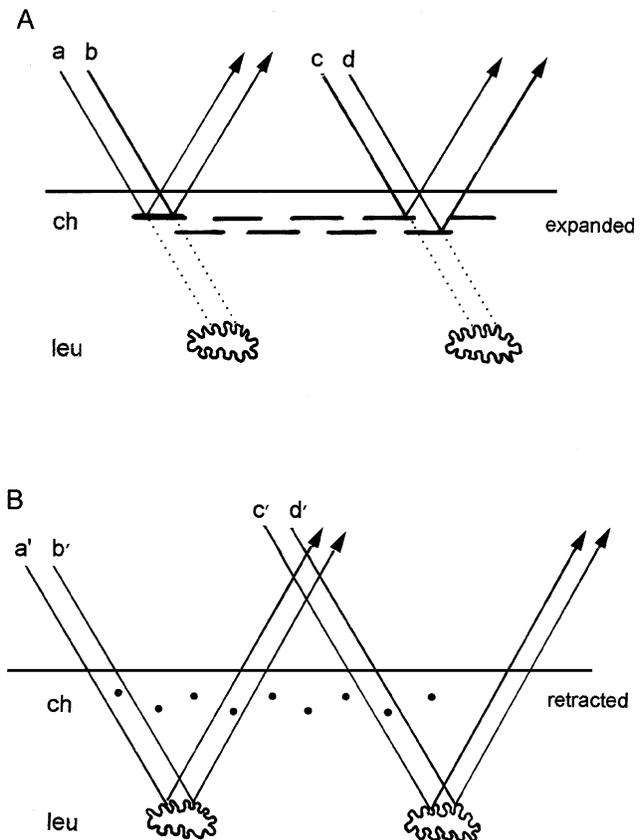


Fig. 38. Role of the leucophores in colour matching in *Octopus vulgaris*. (A) In dim light, the chromatophores (ch) expand and act as a neutral density screen of the background; ambient light rays (a–d) do not reach the deeper-lying leucophores (leu). (B) In bright light, the chromatophores retract (to maintain the brightness match), allowing the ambient light to reach the leucophores (a'–d'): it will then be accurately reflected, whatever its spectral characteristics.

differences in chromatic behaviour. Such differences can be very useful for taxonomists, in that they can help differentiate between sympatric species that otherwise look alike (Hanlon, 1988). The differences are usually associated with different habits (see Hanlon & Messenger, 1996). Shallow-water forms have the most complex body patterns, for either concealment or communication (Section VII), and as a corollary their chromatophores are generally smaller and more densely packed (Table 1). Curiously, some nocturnal cephalopods studied in the laboratory (e.g. *Euprymna scolopes*) have a surprisingly rich repertoire of body patterns, possibly for signalling. At the other extreme, some midwater cephalopods have few chromatophores, reduced chromatophore lobes and presumably few body patterns (Maddock & Young, 1987). The bathypel-

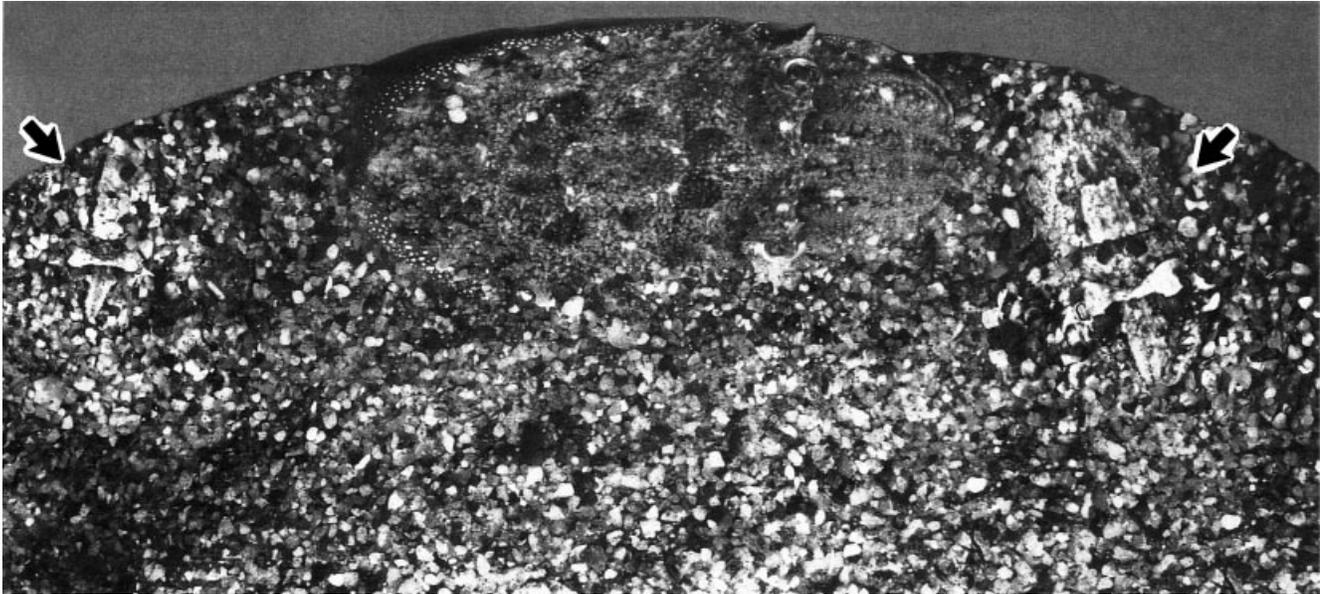


Fig. 39. Differences in patterning response according to age and size. Montage of three individual cuttlefish on a background of small stones: left (arrow), hatchling (mantle length, 10 mm); centre, late juvenile (35 mm); right (arrow), early juvenile (18 mm) (Hanlon & Messenger, 1988).

agic *Vampyroteuthis infernalis* has no chromatophore lobes and its chromatophores lack muscles (Young, 1977a).

VII. FUNCTION: THE LIVING ANIMAL

The chromatophores – and the body patterns that rely so heavily upon them – are thought to have evolved to serve two functions: concealment and communication. A key feature in the evolution of the cephalopods was the reduction and internalisation of the cumbersome shell as they abandoned a sluggish existence on the seabed for an active life in midwater, competing with the vertebrates (Packard, 1972). The loss of a shell may have conferred agility but the consequent vulnerability to sharp-toothed predators must have put a premium on acquiring a sophisticated camouflage mechanism: thus the chromatophores may have evolved primarily for concealment. However, a system of neurally controlled chromatophores is supremely well adapted for signalling and many shallow-water cephalopods also use the chromatophores to make visual signals, both interspecific and intraspecific.

(1) Concealment

In this section the arrangement essentially follows that of Cott (1940), whose classic book *Adaptive*

Coloration in Animals remains invaluable to students of camouflage. It is impossible to convey the range, complexity and beauty of cephalopod body patterns in their environment without photographs, and the interested reader is directed especially to the illustrated articles of Moynihan & Rodaniche (1982), Hanlon, (1988), Hanlon & Messenger (1988, 1996) and Roper & Hochberg, (1988). For this reason the present account of this aspect of chromatophore biology is a summary one.

(a) General background resemblance

An octopus or cuttlefish can conform to the appearance of its background in brightness, colour, pattern and texture. Brightness matching is achieved with the chromatophores, which act, as we have seen, like a half-tone screen. Several examples of brightness can be seen in Hanlon & Messenger (1988): see also Fig. 42. Colour matching is achieved with the chromatophores, iridophores and leucophores. Red, orange and yellow chromatophore pigments can match the longer wavelengths in the environment; and structural greens, cyans and blues can be produced by the iridophores. However, it is the leucophores that, being broad-band reflectors, can reflect incident light of whatever wavelength over the entire spectrum, when the chromatophores are retracted (Fig. 38), and they are believed to be crucial for effective colour matching by colour-blind octopuses (Messenger, 1977, 1979a).



Fig. 42. Neurally controlled polymorphism ('polyphenism'). *Sepia officinalis* hatchlings (mantle length, 10 mm) selecting different body patterns (Stipple, strong Disruptive) on the same background (Hanlon & Messenger, 1988).

In matching the patterning of the background the neurally driven chromatophores come into their own, enabling the animal to conform closely to a variety of backgrounds. Thus the Uniform Light,

Stipple and Mottle body patterns of the cuttlefish enable it to blend visually with sands and gravels of different coarseness. Moreover, the animal is not only able to estimate the degree of graininess of its

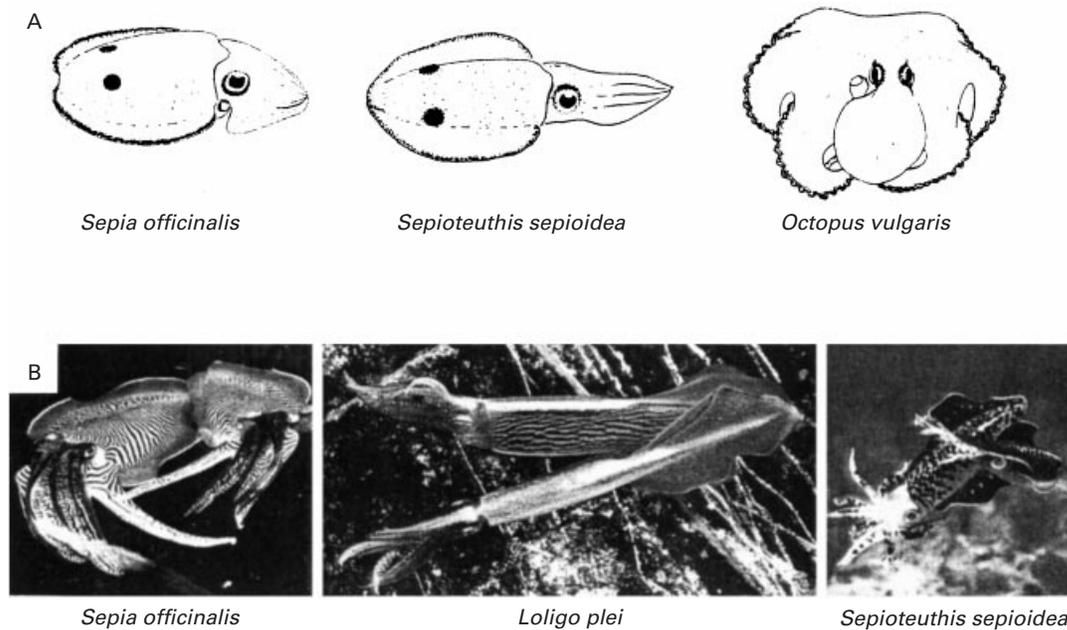


Fig. 43. (A) Interspecific signalling: the strikingly similar Deimatic body patterns show by members of three different orders of cephalopod. (Modified slightly from Packard, 1972). (B) Intraspecific signalling: agonistic encounters in a cuttlefish and two squids (all adults) (Hanlon & Messenger, 1996).

surroundings but also to take into account its own size when selecting an appropriate body pattern (Fig. 39; Hanlon & Messenger, 1988; see also Chiao & Hanlon, 2001). Matching the texture of the background (Fig. 36H) is the function of the skin papillae musculature: as we have seen this is also regulated visually.

(b) Countershading

Countershading in animals is widespread and cephalopods are no exception. On the ventral surface the chromatophores are generally sparse, sometimes with iridophores to enhance reflection; dorsally the chromatophores are much more numerous and tend to be maintained tonically expanded.

More remarkably, however, cephalopods can maintain countershading when they become disorientated. The 'countershading reflex' (CSR) ensures that chromatophores on the ventral surface of the entire body expand when the animal rolls over on its back: a half-roll elicits expansion of the chromatophores only on the upper half of the ventral body (Ferguson & Messenger, 1991) (Fig. 40, Plate 2). There is a CSR in *Loligo vulgaris*, *Octopus vulgaris* and in *Sepia officinalis*, where it is mainly driven by gravity receptors on the maculae of the statocysts (Ferguson *et al.*, 1994). Such a response is, of course, only possible in an animal whose chromatophores are neurally controlled.

(c) Disruptive coloration

On variegated backgrounds a cuttlefish will adopt the Disruptive body pattern, whose effect is to uncouple visually a part of the body from the rest, breaking up the 'wholeness' of the animal (Fig. 39; see also Fig. 42). The fact that cuttlefish only show disruptive patterns when on non-uniform backgrounds was exploited by Marshall & Messenger (1996) in their experiments to show that this species is colour-blind.

Disruptive coloration is a concealment technique widespread among animals (Cott, 1940) so that not surprisingly it is known in many cephalopods. *Octopus vulgaris* has its conspicuous Frontal white spots (Packard, 1988*b*); loliginid squids regularly show transverse dark bands around the mantle that render the animal less conspicuous, at least to human observers; *Sepioteuthis sepioidea* has many disruptive components; and the harlequin octopuses, such as *O. chierciae*, have bold black-and-white stripes and spots (Fig. 41, Plate 2; for references see Hanlon & Messenger, 1996).

(d) Deceptive resemblance

This is Cott's (1940) term for the resemblance of an animal to an inanimate object in the environment. Small cuttlefish often look like stones (Fig. 42) and *Sepioteuthis sepioidea* may resemble, to the human observer at least, floating seaweed, as do other

species adopting the Flamboyant body pattern (see Hanlon & Messenger, 1996).

All these four categories of camouflage technique depend heavily on the chromatophores, but it should be emphasized that they operate in conjunction with the leucophores, iridophores and skin muscles and that appropriate postures are also adopted to enhance the cryptic effect.

Finally, we should recall that although many animals use patterning for concealment (Cott, 1940) it is nearly always a fixed pattern. Cephalopods differ in that their normally controlled chromatophores enable them to select one of several body patterns to use on a particular background (Fig. 42). This is not a trivial point, although its implications have yet to be explored experimentally. For 'rapid neural polymorphism' [or ('polyphenism', as Hanlon, Forsythe & Joneschild (1999a) style it)] may be a significant anti-predator tactic, perhaps serving to confuse the predator's search image through 'apparent rarity' (Curio, 1976); these ideas are discussed more fully by Hanlon & Messenger (1996).

(2) Communication

Cephalopods also use the chromatophores to communicate with other animals, conspecifics or others, and this phenomenon has been studied in detail in several shallow-water species, notably *Sepia officinalis* (Tinbergen, 1939; Hanlon & Messenger, 1988), *Sepioteuthis sepioidea* (Moynihan & Rodaniche, 1977, 1982) and *Loligo plei* (Hanlon, 1982; Di Marco & Hanlon, 1997).

Following Hanlon & Messenger (1996) we can define the final appearance of a communicating cephalopod as a *display* comprising a number of *signals*, the most obvious of which are chromatic; the relationship between signals and displays is thus analogous to that between components and body patterns. Some displays, such as the deimatic display, comprise only a few signals; others, notably some agonistic displays, contain many signals (Fig. 43). Displays, like some body patterns, often rely on elements other than chromatophores for their effect: iridophores or leucophores can contribute significantly to some signals. For example, retraction of specific sets of chromatophores will maximise reflectance from associated leucophores to create conspicuous light spots or bands (Fig. 36G).

(a) Interspecific displays

Cephalopods may make displays when encountering

prey or predators. Thus cuttlefish confronted with a prawn may show the Passing Cloud display (dark waves moving across the body from mantle tip to arm tips) or signal with dark arms (first and/or second pair of arms waved from side to side). The message is presumably 'stop and watch this', drawing attention away from the tentacles about to be ejected (Messenger, 1968; Hanlon & Messenger, 1996). Passing Cloud is also sometimes shown by the common octopus to a crab, perhaps carrying the message 'move, you other animal!' which will make it more vulnerable to attack (Packard & Sanders, 1969).

Displays to potential predators are also common and one particular display is remarkably similar in all three major orders of cephalopods (Fig. 43A). This is the threatening or frightening display that was originally termed 'dymantic' (Young, 1950) but which is now known as deimatic (Maldonado, 1970; see also Hanlon & Messenger, 1996). In its fullest expression, a deimatic display involves the animal spreading and flattening, paling itself centrally but darkening peripherally, creating dark rings around the eyes and dilating the pupil and, in sepoids and squids, creating large dark eyespots on the mantle. The effect of its sudden appearance is extremely startling to a human observer: in particular, there is an apparent increase in size, which is common to the many variants of the deimatic display found in different cephalopods (see Hanlon & Messenger, 1996).

In a very interesting paper, Moynihan (1975) makes the important point that this stereotyped and ritualised display is highly conservative, in that it occurs in such a similar form in sepoids, teuthids and octopods, which diverged at least 200 million years ago. He suggests that this may be because it has been designed for communication to a variety of receivers, all potential predators.

The deimatic display is only one type of display shown during deimatic behaviour; another is the Flamboyant display (Packard & Sanders, 1969), which is characterized mainly by postural signals. This is also very similar in cuttlefishes, squids and octopuses (Moynihan, 1975; Packard & Hochberg, 1977).

(b) Intraspecific displays

Some examples of these are shown in Figure 43B. The most familiar, perhaps, is the Intense Zebra display of the European cuttlefish, *Sepia officinalis*, first described by Tinbergen in 1939 (Fig. 36G).

Sexually mature males commonly show this to females or, during agonistic encounters, to other males.

The display is characterized by a number of features found in other cephalopod displays, both intra- and interspecific: it is highly conspicuous; it is intensity modulated (high contrast black-and-white); it includes a series of stripes (other cephalopods show bars, bands or lines); it includes circles (here white spots on the arms and a dark ring around the eye); the arm posture is exaggerated; and the whole body of the transmitter is oriented towards the receiver. Some of these properties can be obviously be interpreted as devices to maximise the effectiveness of signalling; others may relate to the visual properties of this species, for example its presumed colour-blindness (Marshall & Messenger, 1996). It has been suggested (Packard, 1972, 1988*b*) that the use of circular signals in interspecific displays relates to the presence of centre-surround receptor systems in the eyes of their vertebrate predators: their occurrence in intraspecific displays suggests that such systems may be present in cephalopods, too, as well as line detectors.

The use of the Intense Zebra display by female cuttlefish has also been reported recently (Boal, 1997): again it appears to be used for agonistic signalling, and in this context it is interesting that females are clearly less attracted to males displaying Intense Zebra. This display also appears more often in cuttlefish that are kept in crowded laboratory tanks, and may also indicate stress (Boal *et al.*, 1999).

The displays of some loliginids (*Loligo plei*: Di Marco & Hanlon, 1997) and octopods (*Octopus horridus*: Young, 1962; *O. cyanea*: Wells & Wells, 1972) also involve black and white banding or striping highly conspicuous to human observers.

What kinds of messages are transmitted by cephalopods to conspecifics *via* their chromatophores? So far, we know only the more obvious categories, such as ability and motivation during reproductive encounters, including signalling competence to mate and fighting ability. For example, male loliginid squids give signals to females that appear to say 'court me!' or 'stay near!', while to other males they say 'keep away!' or 'I am stronger, fitter!' (Hanlon & Messenger, 1996). Since the latter signal often involves an apparent increase in size and 'fierceness' cephalopods use 'dishonest' as well as 'honest' signals as do so many other animals (Zahavi, 1987). Cephalopods are such complex and behaviourally advanced animals, however, that they

must be able to transmit messages of a far subtler kind and it seems likely that future work will reveal this. One interesting recent account, for example, suggests that not only may a signal in cuttlefish have two functions simultaneously, but also that a cuttlefish may be able to signal its motivational state (Adamo & Hanlon, 1996).

In this species one signal contributing to the Intense Zebra display – 'dark face' – can be modified to be significantly paler than the other signals, and it has been established that in male-male encounters paler-faced males are less likely to escalate any contest to physical contact and fighting. Such individuals are apparently simultaneously sending two messages to the adjacent male: 'I am a male (so don't copulate with me!)'; and 'I am going to back down' (Adamo & Hanlon, 1996).

But, however subtle this kind of communication, especially for an invertebrate, others have made even greater claim for these animals. Moynihan & Rodaniche (1977, 1982), who report beautiful field data on the Caribbean reef squid, *Sepioteuthis sepioidea*, and show the richness of its chromatic signals and the undoubted complexity of its various displays, speculate that these animals possess a 'language'. Arguments against this somewhat extravagant claim are set out in Hanlon & Messenger (1996) and need not be repeated here, but this is an accessible species deserving further investigation, not least because the study of such an advanced signalling system in a non-vertebrate may help to establish general principles of visual communication.

Finally, mention must be made of the very interesting communication of Shashar, Rutledge & Cronin (1996), who have demonstrated that the cuttlefish *Sepia officinalis* can produce conspicuous polarization patterns on the arms and head (Fig. 44, Plate 4). These patterns are almost certainly produced by iridophores, but the fact that they can be switched on or off instantly suggests that overlying chromatophores are involved in this display. Shashar *et al.* (1996) also demonstrated that cuttlefish react differently to their reflection in a mirror if a special filter deliberately distorts the polarization pattern in it. This implies that cuttlefish, like squids and octopuses, are sensitive to plane polarised light and use polarized patterns for intraspecific communication, a finding all the more significant since such patterns must almost certainly be invisible to most of their vertebrate predators, be they fish or mammals.

The discovery that a loliginid squid (*Loligo pealei*) also shows polarisation patterns on the arms that can somehow be rapidly changed suggests that this form

of signalling may be widespread in cephalopods (Shashar & Hanlon, 1997).

(c) *Special advantages of signalling with chromatophores*

The neurally controlled chromatophores of cephalopods are especially well adapted to signalling for several reasons: (i) the system allows for rapid signalling and for rapid exchange of signals; (ii) signals can be graded in intensity, perhaps even allowing 'emotions' to be expressed (Moynihan, 1985); (iii) simultaneous bilateral signalling is possible. In Fig. 45 (Plate 4), a male Caribbean reef squid is showing a Lateral Silver Display on its left-hand side that repels approaching males while showing a dark display on its right side to maintain attraction to a female; (iv) as the chromatophores are completely independent of the other body muscles signalling does not interfere with other motor acts.

(3) Other chromatic behaviour

Cephalopods are highly evolved, behaviourally active animals that interact with their environment in complex ways, often unpredictably (Packard, 1972; Hanlon & Messenger, 1996). It should come as no surprise, therefore, that their chromatic behaviour is not always completely predictable, and it is simplistic to think of a cephalopod as being in either full concealment or full signalling mode throughout its life.

During attacks on crabs, *Octopus vulgaris* often show abrupt and conspicuous changes of body patterning that are not related to the substrate and cannot always be interpreted as signalling. Warren *et al.* (1974) report similar findings for *O. rubescens* (Section IV) and colour changes during attack on prey, whose function is not always apparent, are also documented for *Sepia officinalis* (Messenger, 1968).

Although octopuses generally conceal themselves from predators in broad daylight, there are many reports from divers of individuals wearing conspicuous body patterns where it would seem to be inappropriate (R. T. Hanlon, personal communication). The recent field study of Hanlon *et al.* (1999a) of *Octopus cyanea* in shallow reefs in the Pacific is particularly interesting in this respect, for it recorded the times spent by octopuses in 'highly cryptic', 'moderately cryptic' or 'conspicuous' body patterns. Surprisingly, they were logged as 'conspicuous' for as much as a quarter to a half of the total observation period, although they must have been potentially vulnerable to attack in the clear reef

waters. An even more surprising finding of this study was the frequency with which these octopuses changed their appearance during foraging: nearly three times each minute over several hours. The significance of such findings, which reflect the fact that the chromatophores are under direct nervous control, remains to be explored, however.

VIII. THE CEPHALOPOD SKIN AND ITS DESIGNERS

If it is reasonable to assume that the chromatophores (iridophores, leucophores and papillae muscles) evolved for protection against predators (Section VII), we have to ask which predators? Living cephalopods are preyed on by a variety of animals: other cephalopods, toothed whales, pinnipeds, birds and, of course, fish, both sharks and teleosts (see Hanlon & Messenger, 1996). In the Mesozoic seas, too, their major predators were probably vertebrates.

Although cephalopods arose towards the end of the Cambrian, the coleoids (i.e., the soft-bodied forms that today comprise the great majority of living cephalopods) did not develop until the late Triassic, beginning to radiate in the Cretaceous (see Packard, 1972). The early squids and octopuses thus evolved contemporaneously with the elasmobranchs and teleosts and lived in seas where ichthyosaurs were considerable predators too (Pollard, 1968). One feature contributing to the success of the fish is their visual system: not merely the special lens conferring high acuity over all parts of the retina (Pumphrey, 1961) but small receptors (sometimes in a fovea), neural sharpening in the retina and often colour vision. There seems no reason to underestimate the visual capacities of the extinct marine reptiles either; the eyes of plesiosaurs, pliosaurs and ichthyosaurs will have had good acuity and probably had colour vision.

Thus the selective pressure on the cephalopods to acquire effective camouflage (and so much else: Packard, 1972) must have come from largely from vertebrate predators. The fish visual system contains ganglion cells with the 'centre-surround' organization typical of vertebrates (Nicol, 1989) and, as we have seen, the cephalopod skin is often organized into circular patches, spots or rings. There are also complex bar detectors in the fish optic rectum (Guthrie, 1980) and cephalopods use bars, stripes and bands of different brightness as components of their disruptive patterns. And although most ceph-

alopods are probably colour blind, and communicate intraspecifically by visual signals couched in terms of brightness contrast, several shallow-water forms have developed broad-band reflectors (leucophores) to make the skin conform to the background in terms of colour as well as brightness. The effectiveness of cephalopod camouflage to a human observer, even in a shallow, well-lit laboratory tank, is difficult to convey; and underwater observations have shown fishes to be similarly deceived as they swim only a few centimeters from cryptic cuttlefish (Hanlon & Messenger, 1988).

Whether or not it is selection pressure from the vertebrates that has designed the cephalopod skin (and for further discussion of this thesis see Packard, 1988*b*) there can be no doubt that this skin is highly complex, its constituent elements being precisely ordered in three dimensions. This may be morphogenetically costly but the benefits are clear: despite the visual prowess of their many predators, the soft-bodied, vulnerable cephalopods are highly successful and numerous.

IX. CONCLUSIONS: HOW ARE 'COLOUR CHANGES' BROUGHT ABOUT?

(1) Cephalopods can abruptly change their appearance, or body pattern, which involves far more than a change of colour. They do so mainly by virtue of their chromatophores, which are neuromuscular organs innervated directly from the brain, and not under any kind of hormonal control. The chromatophores achieve their visual effects in conjunction with other 'elements' in the skin: iridophores, leucophores and skin muscles. These are organized precisely with respect to each other as a result of complex and highly organized developmental process.

(2) Much is known about the ultrastructure, physiology and pharmacology of the large chromatophore organs of loliginid squids. Their radial muscles contract under the influence of an excitatory transmitter, L-glutamate, acting to cause Ca^{2+} -induced Ca^{2+} -release from stores inside the cell: this expands the chromatophore. They relax partly as a result of elastic forces stored within the elastic pigment sac but also under the direct influence of 5-HT, which acts *via* a second messenger system that leads to a suppression of Ca^{2+} release from the Ca^{2+} stores in the muscle: this retracts the chromatophore. Such 'active relaxation' may explain some of the phenomena seen after nerve section (Packard,

1995*c*). In some cephalopods, peptides are also involved in chromatophore expansion.

(3) Far less is understood about the central control of chromatophores. It has been known for nearly 50 years that there is a top-down hierarchy in the brain:

optic lobes – peduncle lobes – lateral basal lobes –
chromatophore lobes (ACLs, PCLs)

but exactly how this operates is still a matter for conjecture, largely because of the difficulties of recording from the cephalopod brain.

It is usually assumed that, as a result of the visual input, cells in the optic lobe select an appropriate body pattern from the small set of patterns that are 'hard-wired' into the central nervous system: there is no indication that body patterns are learned. The body pattern is realized by chromatophore motoneurons located in the ACLs and PCLs, whose activity, or inactivity, leads to expansion, or retraction, of specific sets of chromatophores in the skin. In *Octopus vulgaris* many PCL neurons are dye-coupled and it seems likely that banks of chromatophore motoneurons act in concert to produce the components (bars, bands, lines) shown on the skin. The role of the lateral basal lobes remains uncertain: they may contain the programs for body patterns or somehow 'fine tune' the activity of the chromatophore motoneurons to which they project, setting the appropriate contrast, degree of expansion and grey tone of the chromatophores. A curious feature of the chromatophore system in the brain is that it utilizes all the classical transmitters, a profligacy that may stem from the need to 'line label' the separate pathways controlling the differently coloured chromatophores.

(4) Cephalopods regulate the chromatophores mainly on the basis of visual information, gathered by large eyes with excellent acuity, form vision, sensitivity to intensity differences and polarised light sensitivity, but lacking the ability to resolve wavelengths (Messenger, 1981). In camouflage mode, octopuses match the brightness of the background by using their chromatophores as a kind of half-tone screen. If the background is sufficiently bright the chromatophores will retract sufficiently to allow light to reach the underlying leucophores, which can then reflect that light to help achieve a colour match. If there are discontinuities in the visual environment – and in the sea this will be often – the optic lobes will select a Stipple, Mottle or Disruptive pattern and activate the appropriate chromatophore motoneurons. The various grades of Disruptive

patterns are especially effective for concealment in these as in other animals, and neurally controlled chromatophores are particularly well suited to generate such patterns.

(5) What the viewer of the cephalopod skin sees (whether a biologist or a barracuda) is the whole body pattern, and it has proved useful to see this as comprising not only chromatic but also textural, postural and locomotor components, each of which is built of units, in turn built out of elements – among which the chromatophores are the most important.

(6) In signalling mode, either to warn would-be predators or attract potential mates, the optic lobe selects bold, simple body patterns, for the signals are invariably framed in terms of intensity contrast rather than colour. The similarity of the deimatic patterns in phylogenetically distant cephalopods suggests that they are very ancient and were designed to communicate to a variety of predators that all shared similar mechanisms of visual perception.

(7) The complexity and beauty of visual signalling in cephalopods call for more fieldwork using modern underwater filming techniques. It may be that the study of communication with chromatophores can teach us something about the general principles of visual signalling in animals.

(8) Finally, we must remember that the chromatophores and their associated elements are part of a larger system, the skin. Many years ago, J. Z. Young (1957) wrote of the mammalian skin:

Being the boundary of the body the skin ‘represents’ the environment in a particularly clear sense. It is able to conform to the outside conditions by its texture, colour, scent, temperature and other features. This conformity is maintained by control on all time scales ranging from the few seconds required for temperature regulation to daily or seasonal changes and the many generations for selection of a new set of genes controlling the coat colour.

Here, Young is exploring the dynamic relation between the outside boundary of an animal and its environment. It is extraordinary how well his analysis applies to the skin of cephalopod molluscs.

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