SIZES AND DISTRIBUTION OF CHROMATOPHORES DURING POST-EMBRYONIC DEVELOPMENT IN CEPHALOPODS

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INTRODUCTION

If we are to discover whether the distinctive patterns of chromatophores (spots) on the head, arms, mantle and funnel of hatchling squids and octopods have any function other than to provide systematists with a god-given means of identifying larval cephalopods in plankton hauls, we shall need to bear two things in mind. 1) Chromatophores are visual effectors under neuro-muscular control: spots flash on and off — by expansion and retraction — and create visual effects tuned to eyes: eyes that have much in common with the systematist’s, eyes for recognizing members of one’s own species and for gathering information about food or for alerting...
to potential danger, and eyes that can be tricked. 2) The initial arrangement of spots influences all subsequent arrangements.

F.G. Hochberg has discovered that hatchling octopods can be simply keyed down to the species (? genus) level by the characteristic number and positions of chromatophores. (Similar criteria were used by McConathy et al. (1980) for distinguishing hatchlings of Loligo and Lolliguncula). It has, however, not been generally realized that these hatchling spots persist throughout the weeks of « larval » life and into the months of benthic life that follow. They do not die or disappear, and thus they furnish an invaluable way of linking early planktonic stages with much later stages of the life history the intermediates of which may be completely absent from plankton collections or are difficult to relate using other characters. Octopus vulgaris has a maximum of eight tegumental chromatophores on the dorsal mantle surface at hatching (Fioroni, 1965, Fig. 33) — a forward pair (but sometimes 3 or 4) at the boundary between mantle and head and a posterior pair (but rarely 3 or 4) — yet even after this tiny individual has grown ten-thousand fold in size (from 3 mg body weight to 30 g) these founder chromatophores can still be discerned, embedded amongst the hundreds of thousands of spots that have arisen in the intervening space and time unaltered in size, shape and relative positions (see Plate Ia).

As we shall see, there is a spacing principle at work by which the locations of spots of subsequent generations is influenced by the spots already present. Put another way, this means that chromatophore arrangements are always patterned, never random, and the patterns are spatio-temporal. Pictures through the surface of the skin in which all four dimensions are collapsed into two, offer as much information about the ontogenetic history of the organism as does a section through the trunk of a tree or a scale from a fish, but unlike these the developing structure does not need to be sacrificed in the process.

One very simple kind of pattern resulting from this spacing principle is illustrated in Figure 1a, b of a 1-week-old Sepiola robusta. The new, small, chromatophores (« generation II ») are arranged around and between the larger, older, spots (« generation I »). The best model that I have encountered for understanding these kinds of regular/irregular pattern is Meinhardt and Gierer's (1974) theory of lateral inhibition. It involves activator and inhibitor substances each with its own diffusion range and has been mathematically formulated in two simultaneous equations. Figure 1c shows the locations of

![Fig. 1.](image-url)

a. Arrangement of spots (retracted chromatophores) on the dorsal mantle surface of 1-week-old Sepiola robusta. (From a photograph by J. Lecomte taken for R. Hanlon.) Dorsal mantle length 6.5 mm. b. Size histogram (resting diameters) of spots in this photograph. D. vulgaris (No 4) 12/72 DAYS 3-9 (Ventral) c. Drawing (from photograph) of part of the ventral mantle surface of an early benthic Octopus vulgaris showing new spots (x) that have arisen over a 6-day period in spaces between extant ones (●). Note that some appear in pairs and that extant chromatophores range in size. d. Computer model by H. Meinhardt of regular/irregular arrangement of extant spots (+) and a new generation (x) arising between them, some as a pair or in a row, based on principle of lateral inhibition (see text). Both illustrations first published in Biological Pattern Formation by H. Meinhardt (1982).
new chromatophores arising, some of them as pairs, in a field already occupied by spots. Meinhardt's computer model of this, employing the principle of lateral inhibition, is shown alongside (Fig. 1 d) (Meinhardt, 1982).

I have published the general rules underlying the generation of chromatophore patterns as « Rules for the conduct of young chromatophores » in Packard, 1982. Figure 1 illustrates the rule that young chromatophores should never grow larger than neighbours already established. The resulting hierarchy of sizes reflecting age classes is perhaps the most valuable single key to the correct analysis of chromatophore patterns in cephalopods.

A more detailed example of the operation of the age/size hierarchy and of associated rules is illustrated in Figure 8 (see main text).

METHODS

Animals

Early benthic octopuses (Octopus vulgaris Lamarck) were obtained from local fishermen through the live animal supply service of the Naples Zoological Station and maintained in small black perspex tanks with transparent lids and fed at intervals on Carcinus maenas or the marine isopod Sphaeroma between sessions of photography.

Planktonic (? immediate post-hatching) Octopus dofleini were caught at the night light at the Friday Harbor Laboratories of the University of Washington in December and January initially by Claudia Mills and kindly transported by her to Canada (University of Victoria) where they were kept in circulating seawater. Others were studied at the Friday Harbor Laboratory.

Anaesthesia, photography, measurement

All analyses have been on photographs of the intact skin of live animals working both backwards in time and forwards. Single individuals were followed at magnifications and resolutions sufficient to identify single chromatophores. The photographs that served for analysis of resting sizes and recruitment of chromatophores were taken with a Leica back on a Leica Panphot in the reflecting microscope mode with the lowest power (x 3.8) water immersion lens (illumination mercury vapour lamp) using Kodacolor negative film (ASA 100) and Ektachrome (100 ASA). The anaesthetic used was urethane (ethyl carbamate) 0.5 — 1.0 % in seawater (depending on size of animal). The aim of anaesthesia is to reduce movement and to obtain chromatophores in the retracted (resting) condition. (N.B. I have subsequently found that the popular invertebrate relaxant magnesium chloride (MgCl₂ isotoxic, 1 part + seawater, 1 part) is a better anaesthetic and does not have the long-term toxic effects of urethane). Anaesthetized animals were held (sometimes for up to one hour) in a small bath at room temperature on a soft polystyrene foam bed cut to the shape of the animal and areas to be photographed were either flattened under glass (large microscope slide) or directly by the condenser of the water immersion condenser/objective. Areas of photographic prints and slides to be sampled were inspected either with head lenses or under the x 6 and x 12 objectives of a Wild dissecting microscope and chromatophores were drawn by light tube (camera lucida). Final linear magnifications achieved ranged up to x 70.

GENERAL

The studies of Naef (1921-28) on late embryological stages were extended in detail by Fioroni (1965) for the development of chromatophore patterns (Musterentwicklung). Their data and mine are combined in what follows. Terms are those of current developmental terminology.

Fields

There are four tegumental fields — arms, head, mantle and funnel (Fig. 2) — each with its own polarity and characteristic rates of chromatophore genesis, etc.

Orientation and shape of the fields

Morphogenetic gradients in the arm and mantle fields are initially proximo-distal (i.e. away from the brain) and either dorso-ventral or ventro-dorsal (see Fig. 2). Edge-effects are common especially in early stages of development. As previously empty fields become occupied by recruitment of chromatophores their polarity may invert one or more times. For instance, in Octopus vulgaris at hatching there are many more tegumental chromatophores on the ventral mantle surface (Fig. 2) than on the dorsal, and this condition persists until the “larva” settles from the plankton; but in all benthic stages of this animal there are more on the dorsal mantle surface. The build-up of the gradients producing these effects is illustrated diagramatically in Figure 3. Presumably in this species the early gradients collapse or convert to inhibitory ones once the planktonic phase is over.

Octopus dofleini (Plate 1b) hatches with many tegumental spots dorsally but none ventrally — i.e. the reverse of the situation in O. vulgaris — indicating that reversal of gradients is one of the epigenetic variables that can be played upon by evolutionary processes.
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Fig. 3. Gradients of spot production on the dorsal and ventral mantle surfaces of Octopus vulgaris as indicated by sizes and numbers of chromatophores with time (1, 2, 3) during planktonic life (dashed lines) and early benthic life (dotted lines).

Fig. 2. Hatching dress (Schlüpfkleid) of Octopus vulgaris emphasizing founder chromatophores of tegumental series in the arm and mantle fields (extrategumental spots dotted outlines). Pigment density indicated by shading. a, Expanded, lateral, ventral and dorsal, b, Retracted (left is dorsal, right, ventral). 1 — newly formed anterior and posterior pairs of founder chromatophores at edges of dorsal mantle field. c, Sequence of appearance of tegumental founder chromatophores (full arrows) and of extrategumental series (dotted arrows) during late embryogenesis. (All three figures from Fioroni, 1965, modified).

Plate I. — a, Low power photograph of skin taken by reflecting microscope at left anterior margin of dorsal mantle field of juvenile O. vulgaris (30 g body weight, d.m.l. 46 mm) to show single large chromatophore of original hatching series (“founder” chromatophore) surrounded by populations of smaller chromatophores (see text). Smallest spots (grey) are orange/red in life. All chromatophores in resting condition. Note the “patch” and “groove” arrangement characteristic of these late stages of ontogeny. Densities in the patches are higher, and chromatophores smaller, than in the grooves. Scale bar = 0.5 mm. b, Dorso-lateral aspect of anaesthetized Octopus dofleini caught at the night light showing founder chromatophores on arms and mantle (some of the smaller anterior mantle chromatophores indicated by arrows). Buccal mass, eyes, brain, visceral mass and overlying extrategumental chromatophores all conspicuous, also pallial connectives, stellate ganglia, gills and hearts. Bright points in the dorsal skin of mantle and head are Kölliker’s bristles. Note absence of founder chromatophores on ventro-lateral (and ventral) surface part of which is seen at right of photograph. Scale bar = 1 mm. c, Ventral view of mantle of early benthic O. vulgaris “No. 2” (anaesthetized) analysed in Fig. 6a and 7d (Body weight 0.3 g). All chromatophores in retracted condition. Large ones belong to original hatching series (see text and caption to Fig. 6). Scale bar = 1 mm. d, Ventral view of early benthic O. vulgaris “No. 4” analysed in Fig. 7a and b. Four of the founder chromatophores (1) linked by lines. e, Detail of the middle of the mantle (right side) of specimen “No. 4” showing the extensive population of small chromatophores already recruited during benthic life. Note wide zones of inhibition round some of the “founder” and marker chromatophores (up to 500 μm across, far left and lower right) and tendency of chromatophores to arise in rows. Minimum nearest neighbour distances to founder chromatophores 100 μm). All chromatophores in resting condition. Scale bar = 1 mm. f, Dorsal view of “No. 2” (see c this Plate) under glass anaesthetized. Some of chromatophores expanded, especially in area of skin in contact with glass. Note anterior and posterior pair of large “founder” chromatophores (l) on mantle and conspicuous double row on dorsal (aboral) aspect of arms. Mantle-frontal and arm-white spots (m.w.s., f.w.s. and a.w.s.) and white head bar (h.b.) also well established. g, Same specimen, partially anaesthetized in natural posture, pale (all chromatophores retracted).
Evidence for metamerism and field sub-division, and the effects of the shapes of fields on these processes, are presented in the sections that follow.

**Extrategumental chromatophores**

These very conspicuous spots in the connective tissues overlying the visceral mass, head and eyes are the earliest dorsal chromatophores to arise in the development of *Octopus vulgaris* (Fig. 2) and many other forms, and since cephalopod larvae are so transparent they can often be mistaken for tegumental chromatophores lying in the true skin (see Plate 1b). My studies are not concerned with them, but they often appear in photographs of the mantle and head skin as enormous melanophores orders of magnitude larger than the tegumental spots above them (1). It may be that Joubin’s curious description (Joubin, 1892) of chromatophores as arising by invagination from the surface ectoderm applies to these large extrategumental chromatophores.

In my own mind there is a question over the distinction between extrategumental and tegumental chromatophores at least with regard to the head and arms. The founder series of arm spots (see below) lies deep, on top of the connective tissue surrounding the arm musculature, and appears, whether looked at in life or in light microscopic sections, to be continuous with the extrategumental spots lying on top of the head musculature (see Naef, 1928).

**ARMS**

The arm field, subdivided into 8 (or 10) sub-fields shows the influence that field shape and size has on chromatophore genesis: particularly a) the sequential (proximo-distal) appearance of spots as the arms grow from the tips, b) their serial arrangement in one or more lines down the length of the arms, c) the relative age/size rule (more distal, later, founder chromatophores are smaller than more proximal, earlier, ones), d) the progressive darkening of chromatophores with age, the latest spots (near the tips) being always yellow or orange/red. Details of these and of the pigmentation sequence of the arms during late hatching stages are illustrated for the various cephalopod families by Fioroni (1965).

Hochberg has drawn attention to the systematic differences between octopuses in the linear arrangement of founder spots down the arms of hatchlings (into one or two lines). Figure 4a shows an extreme example: spots in a single line (uniserial) on dorsal arm of *Octopus dofleini* caught at the night light at Friday Harbor Laboratory. As most of the

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(1) Fioroni's (1965) counts, and the mean values given in his tables and figures, always include the extrategumental spots, usually on the visceral mass. In his table 15 and Figure 33, he gives complete details of the numerical distribution of these (range 5-10) along with the arrangements of the posterior two (but sometimes 3 (28%) or 4 (3.8%) of tegumental spots at hatching in a sample of 1116 hatching *O. vulgaris*. Unfortunately he does not say anything about the origin of this large sample, for instance whether they were all from the same brood or not.
specimens caught at the night light have about nine dark chromatophores (the same as the number in Gabe's (1975, Fig. 2) on the first dorsal arms, they are assumed to be freshly hatched. This specimen has 12 dark red spots and 11 orange/red. Most of the orange/red (which will in turn become dark) are the beginning of a second series of spots, smaller than the first, forming a double row. The regression in size of the sequentially produced uniserial spots is shown in Figure 4b.

Figure 4c is a composite diagram from Rees's three Octopus "larvae" in the British Museum. In this species (supposed to be O. vulgaris) the double row is added distally, as the arm grows in length, after four (sometimes only three) uniserial spots. There are 41/2 'pairs' at a ventral mantle length (v.m.l.) of 4 mm and 8 1/2 'pairs' by the late planktonic stage (v.m.l. 6 mm). An even later — immediate pre-benthic — stage is illustrated in Adams (1937). In his drawing, there are four uniserial spots on Arm 1 left and three on Arm 1 right. The double row on Arm 1 left consists of 13 pairs of spots.

The long double row is still conspicuous on the arms of benthic stages of Octopus (Plate Ia, f and g) and can be traced well into juvenile life so long as the skin is pale (overlying chromatophores retracted). While evidently in two rows (biserial), these founder spots are usually unevenly staggered and therefore not really in pairs. Subsequently appearing spots are also shown in Figure 4c including a distally running ventral (oral) series in two single lines just above the suckers (shown on the medial side only in Fig. 4c). The members of these two oral rows are smaller, but not much smaller, in size than their companions aborally and their spacing indicates that they belong to the same generation as the aboral series produced as a result of the extension outwards (from the middle of the arm sub-field), of the same morphogenetic influences that gave rise to the initial double row, directly paralleling what happens to the mantle field at this same stage (see above and Fig. 3). Fig. 4c (3) also shows the first members of a proximal series in a single row but smaller in diameter and closer together than the others, belonging to a subsequent generation.

Nearest neighbour distances between founder chromatophores on the arms are typically 0.15 mm expanding to 0.25 mm by the benthic stage as the arms and skin grow.

MANTLE

On the mantle, as on the arms, the original hatching pattern of chromatophores, or "Schlüpfkleid" (= literally hatching dress, Fig. 2) can still be seen in benthic Octopus showing through the later dress. The spots composing the original dress are identifiable because they are larger in resting size than any subsequent sets of chromatophores, and as the mantle grows throughout its surface (and not terminally like the arms), they retain, by and large, their original positions (i.e. configuration) relative to each other and to the mantle fields as a whole.

Ventral mantle

In Figure 5a I have identified the 16 founder chromatophores (resting diameters ≥ 70 μm) of the original hatching dress on the ventral mantle surface of an early benthic O. vulgaris, and an adjacent drawing (Fig. 5b) shows them as they would have appeared in this specimen when it hatched. The figure also illustrates the genesis of spots. Founder chromatophores are shown alone on the left side of Figure 5a, and together with subsequent generations on the right. A photograph of this animal appears as Plate Ic.

The mantle field of Octopus exhibits edge-effects...
but no obvious proximo-distal sequence or serial arrangement of spots presumably because the mantle field, unlike the arms, is broad and short. Squids such as _Allooteuthis_ (see Fioroni 1965) and _Illex_ with long narrow mantle do show serial arrangements down the length of the mantle. Nevertheless in _Octopus_ spots tend to cluster and to arise in rows, presumably as a result of activator and inhibitor influences emanating from extant chromatophores of the kind mentioned above. The tendency to form rows was noticed by Fioroni (1965), who shows various arrangements on the ventral surface of _O. vulgaris_ at hatching (his Fig. 32 and Table 14); it persists into later stages of ontogeny and can be detected in Plate 1c. Clustering of new chromatophores is revealed in the analysis (Fig. 6a) of the recruitment of spots into the ventral skin of an individual (seen in Plate 1d and e) over a 6-day period. During this period the total population of chromatophores grew by just over 10% (2). As explained in the caption, nearly half arise in the neighbourhood of extant founder and marker chromatophores; they are, however, distanced from them by a minimum nearest neighbour distance of 100 μm again suggesting lateral inhibitory influences.

Rates of chromatophore production evident in these analyses are higher laterally (and posteriorly) than in the middle of the ventral surface (where they reach zero). They are part of the outward extension of the large wave of chromatophore production that appears in the middle of the dorsal mantle field at the transition from planktonic to benthic life (Fig. 3).

**Dorsal Mantle**

The dorsal mantle field is more complex than the ventral. Until the post-planktonic wave appears, chromatophores on the dorsal surface seem to owe their production to an extension (laterally and posteriorly) of the wave (Fig. 3, dashed lines) that originates midventrally in the late embryo and continues to operate during planktonic life (see also Adams' (1937) drawings and photographs but omitting the large dorsal extrategumental spots on the visceral mass). When _Octopus vulgaris_ settles from

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Fig. 6. — a, Chromatophore genesis on ventral mantle surface of early benthic _Octopus vulgaris_ (drawn from photograph). The incidence of new spots (o) is shown in relation to founder and other marker chromatophores (●) on the anterior seven eighths of the left mantle surface of an 0.3 g body weight ("No. 4") _O. vulgaris_ between days 3 and 9 of observation. The circles drawn round founder and marker spots cover a total of 15% of the area analysed. 43% of new chromatophores fall within these circles. Note also "edge effects" at anterior mantle margin. Area analysed approximately 17 mm². Total number of all chromatophores in this area on Day 3 was 580; total number of new by Day 5 was 52 (5.5% increase in two days) and by Day 9 was 61 (10.5% increase in six days). Sample specimen as Plate 1c. b, Relationship of mantle founder chromatophores (anterior 4 and posterior 2 indicated by arrows) to other features of skin patterning in an early benthic _O. vulgaris_. Boundary between head and mantle fields indicated by dashed line. The diamond-shaped group of four primary long _papillae_ (l.m.p.), or cirri, is linked by lines; lateral and posterior long papillae indicated by the Roman numeral I. Secondary and tertiary papillae (II & III) also indicated. Note relationship of these to mantle white spots (m.w.s.). h.b., head bar (from photograph).
the plankton, the rate of chromatophore genesis into the otherwise empty dorsal mantle field rapidly overtakes the rate of recruitment into the ventral field, with three results: i) chromatophores are smaller dorsally than ventrally, ii) local densities become and remain much higher dorsally than ventrally, iii) the progressive decrease in the size of individual chromatophores with each generation (age/size rule) produces more members in the different size classes dorsally than ventrally, particularly in the smallest size class. The dorsal spurt in chromatophore genesis at the end of the planktonic phase is so dramatic as to hint at something like metamorphosis. It is as if the skin were waiting for its owner to settle on the sea floor before bringing out the fine-grain dress that is going to serve for the rest of its life, and replace the coarse-grain set of extra-tegumental spots (on the surface of the viscera) that served during the transparent planktonic phase. I have not been able to follow the initial details of this process. It has already begun by the time the earliest benthic stages become available as occasional catches in dredges or on the nets of fishermen. But I have analysed the size-histogram for expanded chromatophores in the anterior third of the dorsal mantle field of the earliest stage available to me: a specimen of 0.25 g body weight (DML 8 mm). All tegumental chromatophores in the area were still immature (i.e. orange or red, not brown or black) and in the same state of expansion. The size spectrum and accumulated totals of successively smaller size categories are shown in Figure 7a & b. The latter curve gives the putative increase in the population of spots with age in this part of the mantle (see Discussion and Conclusions).

As mentioned in the Introduction all the founder chromatophores of the original hatching dress can still be seen during these and later stages. In Plate II there are six, four anterior (resting diameters ~100 μm) and a posterior pair (resting diameters ~140 μm). The spectrum of sizes of mature (dark) chromatophores, and their red/brown precursors, in a specimen of similar stage has been analysed for an area in the anterior mantle field (Fig. 7c). The histogram has the same shape as Figure 7a and analyses a similar population of spots, but now in the mature (and resting) condition.

Figure 8 shows the typical spatial arrangement and behaviour of mature and immature spots over a 14-day period of development: i.e. during the period of recruitment represented by the left-hand end of the histograms. Although the figure shows less than 0.2 mm² of the original skin surface, it illustrates all the main rules of pattern generation given in this paper (see caption). They are rules that apply not only to the dorsal mantle surface but, with modifications of the variables, to all parts of the skin.
skin. As far as is known they continue to operate throughout ontogeny (3).

When looking at the array of relaxed or uniformly expanded chromatophores in an area of skin — whether of an octopus or a squid — it is perhaps easier to accept the finding that the smaller chromatophores are the latest (and youngest) arrivals on the scene than to use this knowledge to read the ontogenetic time dimension embedded in the spectrum of sizes that each scene contains. In principle it is possible to do this simply by looking from a distance, or as it were with fuzzy spectacles, and not resolving in the scene chromatophores below a certain size. The lower his resolving power the further back the viewer goes in ontogenetic history. In Plate I f and g, and Plate II, the only individual spots easily resolved on the mantle by the eye at a distance of 1 metre from the photograph are the anterior and posterior pair: i.e., the only tegumental chromatophores present at hatching. At normal reading distance the next generations can be perceived, while the most recent generation require a lens or are apparent only as grain in this reproduction. I have mimicked the “fuzzy spectacles” process (of selected size resolutions) in the analysis given for ventral chromatophores of one of these specimens (Fig. 5). Figure 7 d gives the size-histogram (resting sizes) of the first two generations of ventral chromatophores in this specimen: corresponding early generations of spots in the size-histogram for the dorsal surface (Fig. 7 a, b) are tentatively assigned Roman numerals.

Relation of mantle to other features of patterning

Finally, as chromatophores are part of a larger system consisting of other features (or components) of body patterning (see Packard & Hochberg, 1977) — notably of papillae raised by dermal muscles and of white spot areas underlain by leucophore material — I show the spatial relationship of these to founder chromatophores (arrowed) on the dorsal mantle surface of an early benthic octopus (Fig. 6b). There is also an epigenetic relationship between chromatophores and white spot areas (see Discussion).

It is interesting to note that although papillae can be raised and lowered and chromatophores be switched on and off to produce nervously coordinated body patterns at this stage, in none of my photographs are the founder chromatophores expanded. In Plate II g the specimen figured in this plate is wearing a dark mottle produced by expansion of chromatophores but it is notable that neither the founder chromatophores (anterior and posterior) nor the second generation (marker) chromatophores are activated. Although they persist — and are assumed to remain functional — perhaps these spots inherited from the planktonic phase only take part in patterns when the animal is swimming and not in benthic camouflage patterns.

HEAD

I know nothing about the head field, except that it is different from the other fields — e.g. chromatophore densities in juveniles are twice those of the other fields — and do not understand either its polarities or innervation.

DISCUSSION AND CONCLUSIONS

I have referred both to “generations” (characterized by size and by the stage at which they appear) and to “waves” of chromatophore production. Early on, the spectrum of sizes is discontinuous (as in the Sepiola illustrated in the Introduction) suggesting waves or bursts of production. The wave that produces the founder chromatophores on the ventral mantle surface starts in late embryogenesis (Stage XVI of Naef) at the anterior midline and travels

Plate II. — Pattern differentiation in early benthic Octopus vulgaris. All photographs of the same specimen (“No. 11”) showing developmental changes in mantle dress from transparent condition at first settling (Day “0”) to the typical benthic condition two weeks later. Youngest chromatophores difficult to distinguish from grain in these photographs. a, ventral, day “0”; b, dorsal, day “15”; c) dorsal, day “0”; d and g, dorsal, day “15”; e, dorsolateral, day “0”: f, ventrolateral, day “11”. Also shown, examples of physiological pattern generated by differential expansion of chromatophores (g) and i and by papilla-raising before anaesthetic has acted. To aid the eye, spatial groupings of identical chromatophores on central and dorsal surfaces are ringed or linked by lines (A and B). Arrows on dorsal surface indicate founder chromatophores or original hatching dress (4 anterior, one of them damaged, and two posterior in this specimen). Note the differentiation of the mantle white spots (m.w.s.) which are barely discernible on day “0”. (N.B. in b, the anterior edge of the ventral mantle is withdrawn into the mantle cavity and only visible on the right of the photograph). Scale bar in d = 1 mm.
outwards and backwards. It continues after hatching so that many of the chromatophores that result from it arise laterally and posteriorly during planktonic life spreading up on to the dorsal surface. The later spots are smaller than those arising near the centre of pattern generation — as if the power of the wave were subsiding — but since they have the same characteristic spacing (nearest neighbour distances) as the earlier spots I place them in the same size-frequency envelope as the rest of the ventral
founder series. The generation that follows, arising between them, has characteristically different spacing and size. Similar considerations apply to the arms where the wave starts in the middle of the aboral surface proximally and spreads distally and laterally onto the oral surface of each arm. As in other kinds of populations, the waves or (generations) may overlap in time, with a new generation arising while members of the first are still being born. On the arms, which grow terminally, the founder series is still being laid down distally when the next generation has already begun to appear proximally.

All dark chromatophores (melanophores) pass through a pale (yellow or orange/red) phase. And in Octopus all yellow and orange chromatophores that I have followed from birth eventually become dark (melanophores). But I do not know whether this is true of squids, or of Octopus during later ontogeny (after 50 g body weight). In Octopus the rate of darkening varies from one chromatophore to another, depending in some way not yet established, on position. The change in colour of individual chromatophores with age (and associated change in sensitivity to anaesthetics, see footnote 3 above) is particularly interesting in terms of the nervous control of chromatophore patterns.

Obviously the skin expands in size and changes shape during ontogeny — and I have attempted to show the effect of this on the placing of spots between hatching and early benthic stages in Figure 5a and b — but I have not followed changes in size and shape of the fields in any detail for the simple reason that during the days or weeks of early benthic life with which this paper is concerned these changes are small — and given the elasticity of the skin difficult to observe — compared with the large increases in number and absolute densities of spots over the same period. On the dorsal surface, subdivision of the fields begins soon after settling and is well advanced by the time 1.0 g body weight (dorsal mantle length 13 mm) is reached. Subdivision takes the form of a number of circular areas each representing separate pattern-generating centres in which rates of chromatophore production are locally higher than away from the centres. At first appearance these centres are empty and spaced approximately the same distance apart as the founder and marker chromatophores (see Figures). Variations in chromatophore production by only a few per cent can give rise to peaks and troughs in local density and create the “patch” and “groove” arrangement characteristic of all later stages (see Plate Ia and Froesch & Messenger, 1978). During this process there are evidently interactions between chromatophore pro-

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**Fig. 8.** Genesis of chromatophore pattern in anterior dorsal mantle surface of young Octopus vulgaris. Details of positions, shapes, sizes, and colour of individual chromatophores on Day 1 (left) and Day 15 (right) that illustrate the rules of pattern generation in this species. Spots numbered for ease of identification; mature spots (“melanophores”) black; depth of pigmentation of other spots indicated by shading (yellow, unshaded); all spots in retracted condition (except yellow on Day 1). Rules: a, extant chromatophores retain their positions and do not disappear (1-20); b, yellow and orange chromatophores darken with age (11-20); c, new chromatophores (21-25) are yellow and arise in spaces between extant chromatophores (non-random distribution); d, younger chromatophores (11-25) are smaller than older chromatophores (1-10) and the population forms an age/size hierarchy. (Drawn from photographs of anaesthetized specimen weighing 1.4 g).
duction and other pattern-giving elements, particularly iridocytes and leucophores. In the earliest benthic stages I have studied (0.25 g body weight), the Anlagen of the white spots appear as clusters of iridocytes. One of these, which will form the mantle white spot, is just visible as a crescent of iridocytes that will later be followed by leucophore material as the white spot enlarges backwards (compare also Plate II c with Plate II d and f). Rates of chromatophore production in the tissue overlying these and other white spots are lower than in the area immediately in front of the crescent (resulting in local densities twice as high on the proximal side of the boundary as on the distal). Chromatophore genesis is being locally inhibited either by leucophores or by the morphogens that induce white spot development.

It needs emphasising that all the main findings in this paper come from the practice of following single individuals: individual chromatophores in individual animals. The value of this as a technique can not be overstressed. It is the classical comparative method in which the individual serves as the base of comparison with itself at another stage. The amount of fine-tuned information available in a pair of pictures of even a small area of skin — so long as they are of the same area at different points in ontogeny — is illustrated in Figure 8. What I have called “Rules” are derived from observation. During the process of comparison the eye makes predictions none of which have been falsified in hundreds of hours of analysis of such pairs of photographs. The only chromatophores that I have ever seen disappear during ontogeny are occasional damaged ones (broken or oddly shaped).

Having established that, in any one part of the skin, successive generations of chromatophores have smaller resting diameters than their predecessors and are pale when they first arise, it is no longer strictly necessary to take a second photograph at a later stage to predict earlier states of the skin. A single photograph will do.

The general interpretation of the size-frequency histograms that can be constructed from a photograph is shown in Fig. 9. The slope and position of the curve of accumulative totals should be species-specific so long as original dimensions are preserved.

Whether systematists and seagoing and field biologists will learn to use the potential information about the developmental history of an individual embedded in a single photograph of its skin will depend on whether they are prepared to anaesthetize and photograph a specimen before fixing. Once captured on film, the information does not decay.

ACKNOWLEDGEMENTS. — This work was supported by grants from the Royal Society of London (European Fellowship funds), the Moray Fund of the University of Edinburgh and the Carnegie Trust for the Universities of Scotland. Part of it was done while the author was a Landsdowne Fellow at the University of Victoria, B.C., Canada. My thanks for facilities to the Director and staff of the Naples Zoological Station and of the Friday Harbor Laboratory of the University of Washington.

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Reçu le 18 octobre 1985; received October 18, 1985
Accepté le 5 décembre 1985; accepted December 18, 1985