

Disruptive coloration in cuttlefish: a visual perception mechanism that regulates ontogenetic adjustment of skin patterning

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Summary

Among the changeable camouflage patterns of cuttlefish, disruptive patterning is shown in response to certain features of light objects in the visual background. However, whether animals show disruptive patterns is dependent not only on object size but also on their body size. Here, we tested whether cuttlefish (*Sepia officinalis*) are able to match their disruptive body patterning with increasing size of background objects as they grow from hatchling to adult size (0.7 to 19.6 cm mantle length; factor of 28). Specifically, do cuttlefish have a single ‘visual sampling rule’ that scales accurately during ontogeny?

For each of seven size classes of cuttlefish, we created black and white checkerboards whose check sizes corresponded to 4, 12, 40, 120, 400 and 1200% of the area of the cuttlefish’s White square, which is a neurophysiologically controlled component of the skin.

Disruptive body patterns were evoked when, regardless of animal size, the check size measured either 40 or 120% of the area of the cuttlefish’s White square, thus demonstrating a remarkable ontogenetic conformity to a single visual sampling rule.

Cuttlefish have no known visual feedback loop with which to adjust their skin patterns. Since the area of a cuttlefish’s White square skin component is a function of body size, our results indicate that cuttlefish are solving a visual scaling problem of camouflage presumably without visual confirmation of the size of their own skin component.

Key words: camouflage, *Sepia officinalis*, visual sensori-motor system, crypsis, chromatophore, cephalopod.

Introduction

Camouflage is a common and powerful adaptation to visual predation throughout all animal phyla. Several mechanisms of camouflage, or crypsis, are known: general background resemblance, deceptive resemblance, disruptive coloration, countershading and concealment of shadow (e.g. Cott, 1940; Edmunds, 1974). Crypsis through background matching requires that colors, sizes and shapes of the body pattern components should resemble those of the visual background (Cott, 1940; Edmunds, 1974; Endler, 1978; Merilaita, 1998). In disruptive coloration, camouflage is achieved when the animal’s outline is broken up into patches of different size, shape, contrast, color, etc., such that the observer’s attention is drawn away from the animal’s shape, thus decreasing the animal’s chance of being detected (Cott, 1940; Edmunds, 1974; Stevens and Cuthill, 2006). Furthermore, according to Schaefer and Stobbe (Schaefer and Stobbe, 2006), disruptive coloration on an animal’s edge is an effective camouflage method independently of the background. Recent studies have tested experimentally the principles of both background matching and

disruptive coloration and this is an active area of discussion (see Merilaita et al., 2001; Cuthill et al., 2005; Cuthill et al., 2006; Merilaita and Lind, 2005; Schaefer and Stobbe, 2006; Stevens et al., 2006).

Cephalopods (squid, octopus and cuttlefish, Phylum Mollusca, Order Cephalopoda) are unique in the animal kingdom because of their exceptional neurophysiologically controlled skin that allows for rapid changeable coloration, enabling them to achieve dynamic crypsis in a wide range of habitats (cf. Holmes, 1940; Packard and Sanders, 1971; Packard and Hochberg, 1977; Hanlon and Messenger, 1988; Hanlon and Messenger, 1996). Cephalopod skin can vary in pattern, color, brightness/contrast and texture, allowing the animals to produce an overall repertoire of 20–50 body patterns (defined as the total appearance of the animal) that serve a variety of behavioral functions. This changeable repertoire of adaptive coloration is achieved by a system of dermal pigmented organs, or chromatophores, complemented by structural reflectors called iridophores and leucophores (Hanlon, 1982; Cloney and Brocco, 1983; Messenger, 2001).

The cuttlefish's many body patterns used for camouflage can be reduced to three general categories: uniform/stipple, mottle and disruptive (Hanlon and Messenger, 1988). Uniform/stipple body patterns are used to match generally uniform backgrounds. On non-uniform backgrounds, cuttlefish show mottle or disruptive body patterns for crypsis. Mottle patterns consist of alternating, irregularly shaped, semi-round small dark and light patches in the skin that correspond roughly to the size of dark and light objects in the visual background. Emulating the small dark and light objects of the background in its mantle helps cuttlefish resemble the non-uniformity of many natural backgrounds. Disruptive body patterns are irregular patches of different shape orientation (transverse and longitudinal bars, squares and other shapes), contrast and color that serve to distract the observer's attention from the outline of the animal. In the common European cuttlefish *Sepia officinalis* (Linnaeus 1758), disruptive patterning is most commonly characterized by variable expression of five light and six dark chromatic components (Fig. 1). Each component is an independent neurophysiological entity that can be shown singly or in combination with other components in differing strengths of expression (Hanlon and Messenger, 1988). Among these 11 disruptive components, the White square of a cuttlefish (2 in Fig. 1) is bold and often white. Crypsis is achieved, at least partly, because the White square is expressed when other light objects of similar size are in the visual background, thus rendering the animal's White square as a random sample of other light objects in the background.

The present study was stimulated by Fig. 2, first published in fig. 84 of Hanlon and Messenger (Hanlon and Messenger, 1988). It illustrates how cuttlefish of different sizes can adopt different camouflage tactics on the same visual background. The composite photograph shows a hatchling cuttlefish (left), an early juvenile (right), and a late juvenile (center) on the same background of coarse gravel. The smallest animal is showing a strong disruptive pattern, the early juvenile a weak disruptive, and the largest animal a light mottle, even though the pebble size is the same. The dimensions of some of the light and dark components of the hatchling are close to those of the gravel, so that the disruptive pattern is appropriate; the dimensions of the late juvenile are already such that disruptive components, if expressed, would exceed the gravel sizes in the background. This illustrates that for *S. officinalis*, an important feature of concealment on a given background is related to the size of the animal, as they must be able to estimate the substrate particle size so that they show the appropriate body pattern (Hanlon and Messenger, 1988).

Cuttlefish's ability to camouflage provides a powerful biological assay to study the way these animals perceive their visual environment (Hanlon and Messenger, 1988; Hanlon and Messenger, 1996; Marshall and Messenger, 1996; Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005; Mäthger et al., 2006). Chiao and Hanlon developed a non-invasive, quantifiable methodology using computer-generated images to study body patterning in *Sepia pharaonis* (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b). The observation

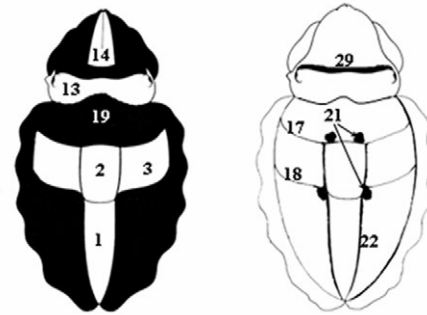


Fig. 1. Diagrammatic representation of the 11 disruptive coloration skin components that are most commonly expressed in the cuttlefish *Sepia officinalis*. These chromatic skin components were used for grading the disruptive body pattern. White posterior triangle (1), White square (2), White mantle bar (3), White head bar (13), White arm triangle (14), Anterior transverse mantle line (17), Posterior transverse mantle line (18), Anterior mantle bar (19), Median mantle stripes (22), Anterior head bar (29), Paired mantle spots (21). The numbers of the components are the same as those used in Hanlon and Messenger (Hanlon and Messenger, 1988).

that visual stimuli lead to changes in body patterns of the skin (motor output) allows detailed analysis of visual perception in cuttlefish. Based on Holmes (Holmes, 1940) and Hanlon and Messenger (Hanlon and Messenger, 1988), Chiao and Hanlon showed that the main visual features that cuttlefish key on for producing disruptive coloration are area, contrast, and density of white elements on a dark background (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b). The same methodology was adopted in subsequent studies in *S. officinalis* to show that disruptive body patterning requires visual information on edges and contrast of objects in natural substrates (Chiao et al., 2005) and to demonstrate behaviorally that they are colorblind (Mäthger et al., 2006) (see also Marshall and Messenger, 1996).

Previously it was found that juvenile *S. pharaonis* consistently responded to white and black checks by showing disruptive coloration (Chiao and Hanlon, 2001a). We have shown that this is also true in *S. officinalis* (Mäthger et al., 2006), but to date ontogenetic variations in this behavior have not been tested. Although hatchling and juvenile *S. officinalis* have been reported to show ontogenetic variations in response to natural substrates (Hanlon and Messenger, 1988; Poirier et al., 2005), this is the first study investigating the disruptive behavior across all developmental stages (from hatchling to adult). Chiao and Hanlon's studies on artificial backgrounds (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b) used only the analysis of the White square component as a measure of disruptive coloration. In the present study, the grading analysis was expanded to 11 body pattern components, and an extensive set of experiments was conducted to study the visual perception of a variety of sizes of light objects (white checks) in the visual background. The present paper demonstrates experimentally that cuttlefish of widely different sizes

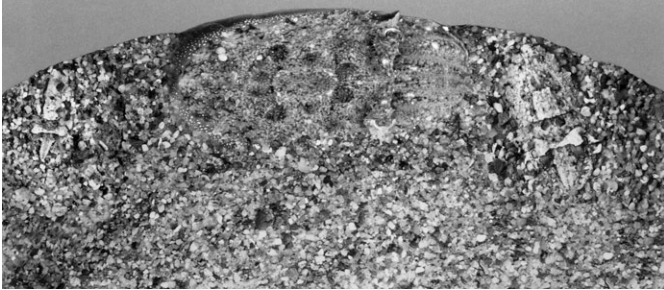


Fig. 2. Montage of three cuttlefish *Sepia officinalis* of different ages (i.e. different body sizes), on the same substrate, showing an ontogenetic shift in body pattern in response to the same visual background [from fig. 84 of Hanlon and Messenger (Hanlon and Messenger, 1988)]. Left, hatchling; middle, late juvenile; right, early juvenile.

apparently use a simple visual sampling rule to account for scaling issues in disruptive coloration.

Materials and methods

Animals and experimental setup

Sepia officinalis L. were cultured from eggs at the Marine Resources Center [see methods published elsewhere (Forsythe et al., 1994; Hanley et al., 1998)]. Seven experiments were conducted from June 2003 to August 2005. All cuttlefish were housed separately for the duration of the experiments. The mantle lengths (ML) and White square (WS) areas of the animals were derived from digital images of the cuttlefish on a known size of white and black checkerboard using NIH Image 1.62 (US National Institute of Health; <http://rsb.info.nih.gov/nih-image/>). For each experiment, six checkerboards with check areas of 4%, 12%, 40%, 120%, 400% and 1200% of the cuttlefish's mean WS area were computer generated, printed and laminated to be waterproof. Check areas were chosen based on a logarithmic scale. For example, for cuttlefish of size class 2, the check areas were 0.01, 0.03, 0.1, 0.3, 1 and 3 cm²; the log of each area

corresponds to an increment of 0.5. Each experiment corresponds to a cuttlefish size class. Table 1 provides all measurements.

To determine the checkerboards' contrast, relative reflectance spectra of check colors (i.e. pure white and pure black, equivalent to 0 and 255 intensity levels, where 0 is black and 255 is white) were measured using a fiber optic spectrometer (USB2000, Ocean Optics, Florida USA). A detailed description of the method used to calculate the apparent contrast as perceived by the cuttlefish eye is published elsewhere (Mähger et al., 2006). Checkerboards' contrast ranged between 84 and 95%.

For experimentation, cuttlefish were placed inside either a rectangular divider or a circular arena with the following dimensions (cm) to accommodate growing cuttlefish: Experiment 1: 9.2×4 diameter×height; Experiment 2: 28×20×11 length×width×height; Experiment 3: 28×20×11 length×width×height; Experiment 4: 24.5×10 diameter×height; Experiment 5: 24.5×14.5 diameter×height; Experiment 6: 38×26×21 length×width×height; Experiment 7: 28×33.5×21 length×width×height. The inside of the divider/arena was lined with black felt. This set-up was placed inside a running seawater tank surrounded by black felt and black plastic sheeting. Animals were allowed to move freely inside the arena and their body pattern was recorded using a digital camera, mounted above the tank. The camera was set to record 2 s every minute, and connected to an external monitor to allow remote viewing to reduce disturbance to the animals and experimenter bias (Boal, 1996). Recording began only when the animals were acclimated. Acclimation (i.e. cessation of excessive swimming and hovering movements and expression of stable body pattern) period was at least 5 min.

Image analysis: grading body pattern responses

Each trial lasted 30 min, yielding 60 s of recorded footage. For analysis, a frame was taken every 6 s from the recorded video footage (10 images per trial), yielding a total of 2720 images for the entire experiment. Eleven skin components of disruptive coloration were graded for each image (see Fig. 1).

Table 1. Cuttlefish body measurements and corresponding checkerboard substrate sizes (expressed as % area of the cuttlefish White square area) used to test pattern ontogeny

Size class	N	ML (cm)	WS (cm ²)	White and Black check areas (cm ²)					
				4%	12%	40%	120%	400%	1200%
1	9	0.86±0.07	0.07±0.01	0.0028	0.0084	0.028	0.084	0.28	0.84
2	12	1.63±0.12	0.25±0.03	0.01	0.03	0.1	0.3	1	3
3	8	3.45±0.21	0.81±0.13	0.032	–	0.32	0.96	–	9.6
4	7	6.87±0.17	3.10±0.33	0.124	0.372	1.24	3.72	12.4	37.2
5	5	10.78±0.86	6.25±0.85	0.25	0.75	2.5	7.5	25	75
6	5	17.47±1.75	22.10±0.53	0.88	2.65	8.84	26.52	88.4	265.2
7	6	18.97±0.47	25.73±4.49	1.029	3.087	10.29	30.87	102.92	308.76

N=number of cuttlefish; ML=mantle length; WS=White square; values for ML and WS are means ± s.d. Animals from size class 3 were not tested on the 12% and 400% check areas.

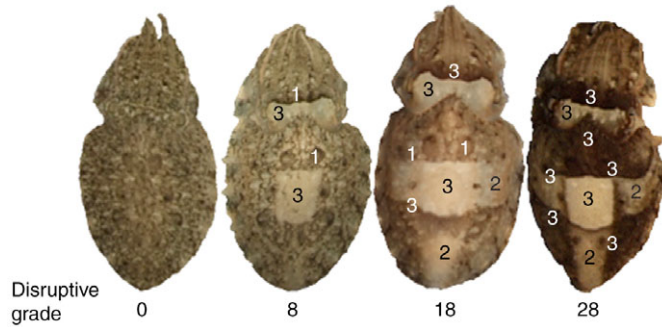


Fig. 3. Sample images of cuttlefish, exemplifying how the 11 disruptive components were graded to evaluate cuttlefish's responses to the different substrates tested. See Materials and methods for further details.

Each component was graded from 0 to 3 (0, not expressed; 1, weakly expressed; 2, moderately expressed; and 3, strongly expressed) (see Fig. 3 for an example of grading). According to this grading scheme, an animal could be given a total grade ranging from 0 (no expression of any disruptive component) to 33 (all disruptive components strongly expressed, i.e. $3 \times 11 = 33$). This method has been used previously (Mäthger et al., 2006). We averaged grades of all 10 images obtained for each animal on each substrate.

Statistical analysis

We found disruptive scores to be normally distributed, and therefore conducted a mixed model ANOVA (Zar, 1999), including a within-subjects factor of check area and a between-subjects factor of cuttlefish size class. We tested if different check areas elicited different disruptive responses, if the different size classes responded differently to the experimental substrates, and finally if there was an interaction between check area and size class. Such interaction would indicate that animals of different absolute size responded in significantly different ways when exposed to substrates scaled equivalently to their White square area. Analyses first included all six substrates and were subsequently restricted to the 40% and 120% check areas. Statistical analyses were performed using SPSS 14 (SPSS Inc.).

Results

Ontogenetic body patterning on checkerboards with different check areas

All size classes showed disruptive coloration on check areas of 40% and 120% of the mean area of the cuttlefish's White square (Fig. 4). When the checks were less than 40% of the cuttlefish White square area, the animals showed mostly mottle body patterns, and on occasion 1–3 disruptive components were expressed, yielding very low scores for disruptive coloration. When checks were greater than 120% of the cuttlefish White square area, cuttlefish showed mostly uniform body patterns.

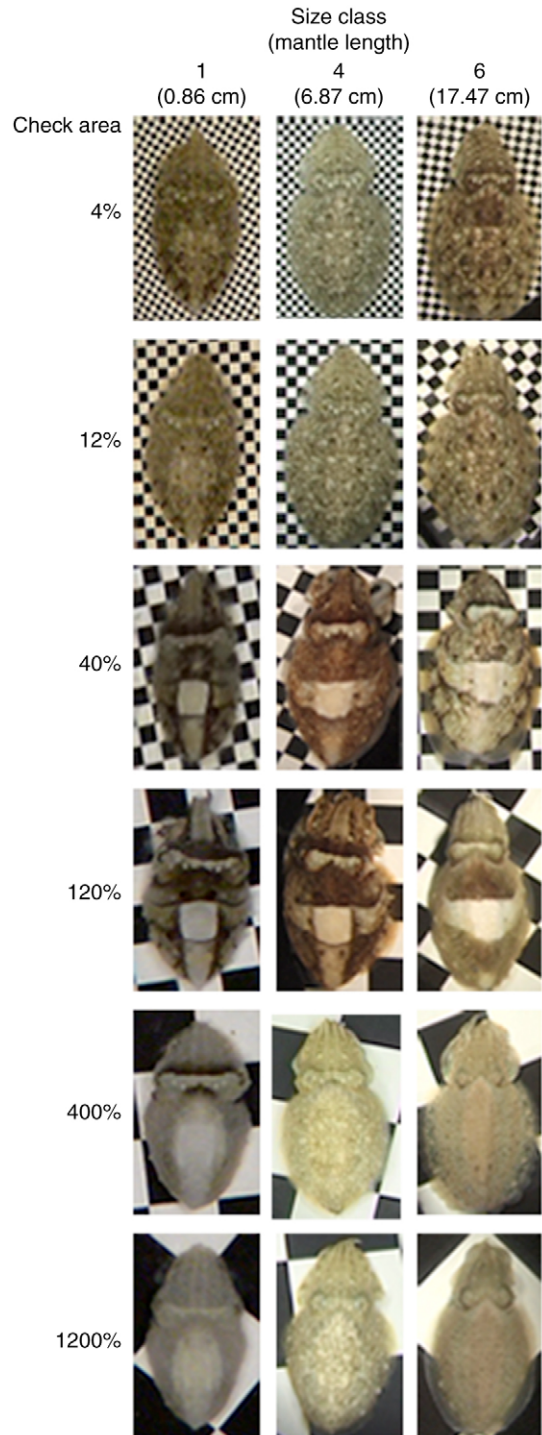


Fig. 4. Disruptive coloration is shown in response to checks that are 40% and 120% of the area of the cuttlefish's White square component. Non-disruptive body patterns (uniform and mottle) are shown on other check sizes. Sample images are from (left to right) size classes 1, 4, and 6. Images are not to scale.

Fig. 5 shows the average disruptive coloration scores of the seven size classes for all six check areas. In all size classes, the highest disruptive scores were recorded on checkerboards with check sizes of either 40% or 120% of the area of the animal's

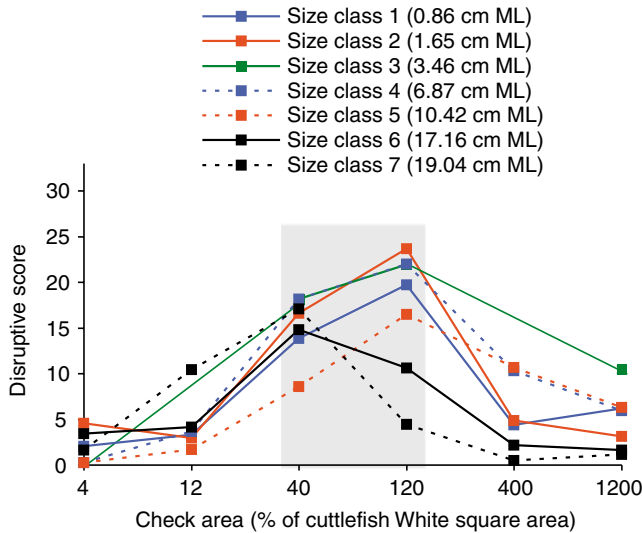


Fig. 5. Average disruptive scores of seven size classes of cuttlefish tested on six check areas (expressed as % area of the mean cuttlefish White square area). Maximal response was evoked at 40% and 120% in animals of all sizes (values inside gray box). Animals from size class 3 were not tested on the 12% and 400% check areas. Error bars are not shown for clarity.

White square (values within gray rectangle). For each size class, there was a tendency for the disruptive scores to progressively increase and then decrease with the increasing ratio of check area to White square area.

A mixed model ANOVA disclosed significant main effects of check area ($P < 0.001$) and size class ($P < 0.001$). Therefore, it is apparent from this result that different check areas evoked different responses by the animals and that the strength of the disruptive response differed among size classes. However, a significant interaction between check area and cuttlefish size class was also detected ($P < 0.001$), which indicates that individuals of the different size classes responded in significantly different ways when exposed to the various treatments (see below).

Ontogenetic variations in disruptive responses to check areas of 40% and 120% of the mean area of the cuttlefish's White square

A glance at Fig. 5 shows that the check area–size class interaction is focused primarily on the 40% and 120% check areas of the mean area of White square. Here we see one of the most notable differences between size classes. Larger animals (size classes 6 and 7) gave stronger disruptive responses on the 40% check area than on the 120% check area, whereas this pattern was reversed for size classes 1–5. To examine this interaction more carefully, we conducted another mixed model ANOVA including only the 40% and 120% check areas as within-subjects factor. As anticipated, the check area–size class interaction was highly significant ($P < 0.001$). Fig. 6 shows the strength of the disruptive response on the 40% and 120% check

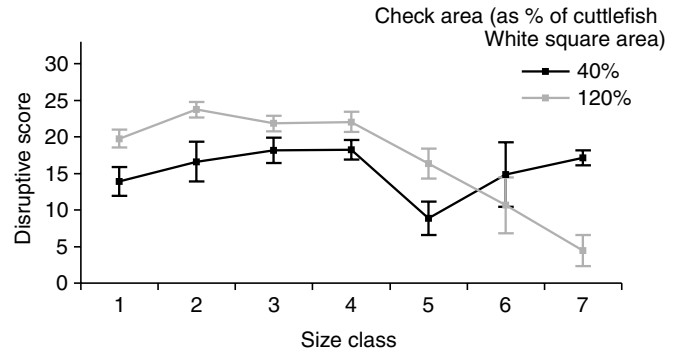


Fig. 6. Average disruptive score of seven classes on the 40% and 120% check areas (expressed as % area of the mean cuttlefish White square area). There are no significant interactions between size classes 1–5; the curves are near parallel. Values are means \pm s.e.m. For size class N , see Table 1.

areas for all size classes. The plot reveals quite strikingly that the interaction is due exclusively to a difference in behavior between size classes 6 and 7 *versus* size classes 1–5. Indeed, size classes 1–5 revealed no internal interactions. The curves for the 40% and 120% check areas are nearly parallel across size classes 1–5, and a mixed model ANOVA restricted to size classes 1–5 and check areas 40% and 120% of the mean area of White square yielded no significant check area by size class interaction ($P = 0.730$).

Qualitative and quantitative differences in the composition of disruptive components

To analyze differences among the expression of the eleven disruptive skin components, we looked at the relative expression of each component on the 40% and 120% check areas (Fig. 7). We grouped the seven size classes in three major groups: small (size classes 1 and 2), medium (size classes 3, 4 and 5) and large (size classes 6 and 7). For each category, we took the mean score of each component and divided it by the sum of all component scores, yielding a final normalized value, with the sum of all normalized values adding to 1. This enabled us to evaluate whether a component was commonly expressed or not, independently of the strength of its expression. The relative level of disruptive components expression differed among groups. The normalized data revealed that the small and medium groups are quite similar in the disruptive components shown. However, the large group produced normalized scores that are significantly lower than those of the other two groups on components White posterior triangle (WPT) and Median mantle stripes (MMS). This same trend holds true if we look separately at the disruptive scores at the 40% or 120% check areas (data not shown).

Table 2 shows the most common combinations of disruptive components expressed on the 40% and 120% check areas, as well as the total number of disruptive combinations shown by each size class. In this table, we only show the four most commonly expressed combinations for each size class. All seven size classes contained individuals who expressed all 11

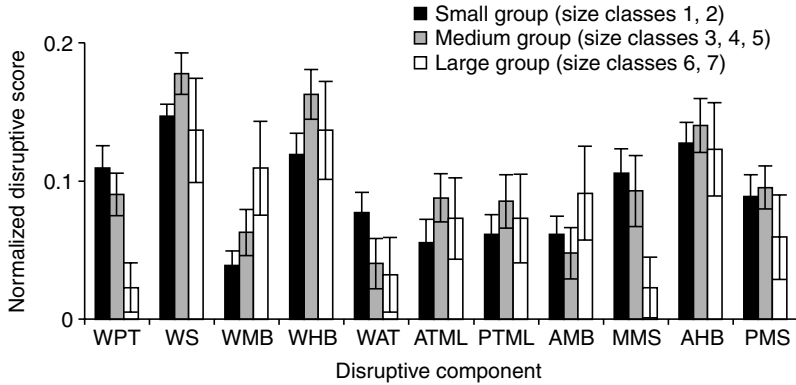


Fig. 7. Normalized data of the 11 disruptive components of the seven size classes of cuttlefish tested on the 40% and 120% check areas (expressed as % of the mean cuttlefish White square area). Cuttlefish size classes were grouped into three groups: small (size classes 1 and 2), medium (size classes 3, 4 and 5) and large (size classes 6 and 7). The relative level of disruptive components expressions differed among groups. Error bars give 95% confidence intervals for the plotted points. Abbreviations are given in the List of abbreviations.

disruptive components (see first row of black squares in Table 2). In all other examples, for which not all 11 disruptive components were expressed, the same disruptive component combinations tended to be expressed among neighboring size classes (e.g. the second row of black squares in Table 2 shows that size class 1 and 2 expressed the same disruptive components combinations while size classes 1 and 7 did not share any combinations). Nevertheless, smaller animals did not exhibit the same combinations of disruptive components as larger animals. Smaller animals exhibited the most diverse combinations of disruptive components (e.g. a maximum of 51 combinations of disruptive components for size class 1, and 49 combos for size class 2).

These results suggest that, although cuttlefish in all size classes can show complex disruptive body patterns, different

tactics seem to be adopted ontogenetically to achieve disruption. This can be seen both in the disruptive components expressed and how strongly they are expressed, as well in the combinations of those disruptive components.

Discussion

These experiments demonstrate that (1) *Sepia officinalis* of all sizes respond to a size-specific cue in the visual background to turn on disruptive coloration, and (2) the size of the cuttlefish's White square is a powerful predictor of the check sizes likely to evoke disruptive coloration in any sized cuttlefish. However, the size of the animal slightly modulates this prediction, with larger animals tending to respond more strongly to slightly smaller-sized checkerboards.

Table 2. Distribution of the four most commonly expressed disruptive component combinations shown by each cuttlefish size classes on check areas of 40 and 120% of the mean White square area

N	Disruptive components expressed											Size class						
	WPT	WS	WMB	WHB	WAT	ATML	PTML	AMB	MMS	AHB	PMS	1	2	3	4	5	6	7
11	■	■	■	■	■	■	■	■	■	■	■	✓	✓	✓	✓	✓	✓	✓
10	■	■	■	■	■	■	■	■	■	■	■	✓	✓					
10	■	■	■	■	■	■	■	■	■	■	■	✓	✓					
9	■	■	■	■	■	■	■	■	■	■	■	✓						
10	■	■	■	■	■	■	■	■	■	■	■		✓	✓	✓			
10	■	■	■	■	■	■	■	■	■	■	■			✓				
9	■	■	■	■	■	■	■	■	■	■	■				✓			
9	■	■	■	■	■	■	■	■	■	■	■					✓		
10	■	■	■	■	■	■	■	■	■	■	■					✓		
7	■	■	■	■	■	■	■	■	■	■	■						✓	
7	■	■	■	■	■	■	■	■	■	■	■						✓	
8	■	■	■	■	■	■	■	■	■	■	■						✓	
8	■	■	■	■	■	■	■	■	■	■	■						✓	
10	■	■	■	■	■	■	■	■	■	■	■							✓
8	■	■	■	■	■	■	■	■	■	■	■							✓
8	■	■	■	■	■	■	■	■	■	■	■							✓

Total number of disruptive component combinations observed: 51 49 34 12 27 19 34

N=number of disruptive components expressed. Black squares represent component expressed. WPT, White posterior triangle; WS, White square; WMB, White mantle bar; WHB, White head bar; WAT, White arm triangle; ATML, Anterior transverse mantle line; PTML, Posterior transverse mantle line; AMB, Anterior mantle bar; MMS, Median mantle stripes; AHB, Anterior head bar; PMS, Paired mantle spots (see Fig. 1 for these skin components). Check marks represent the types of combinations observed. The total number of disruptive combinations is also listed.

Effect of check sizes on body patterning

For each size class of cuttlefish, differently sized checkerboards of high contrast and sharp edges evoked all three camouflaged body pattern types: uniform, mottle and disruptive. The focus of this paper is on disruptive coloration, and the main finding is that checks of 40% and 120% of the mean area of the White square evoked disruptive patterns with noteworthy consistency throughout all sizes of cuttlefish. When checks were much larger than the mean area of the White square, i.e. 400% and 1200%, uniform and some disruptive patterns were evoked. Currently, we do not have a good explanation for the responses to large checkerboards. Responses to larger check areas suggest that the sampled visual field by each cuttlefish eye may be different enough to influence the pattern type. For example, depending on the position on the substrate, a cuttlefish may occupy part of a large white check and may therefore only see a small portion of it, and as a result show disruptive patterning. Conversely, if a fully visible large check predominates the immediate visual field of a cuttlefish, then a uniform pattern might be expected. When checks were small (i.e. 4% and 12% area of the White square) mottle was a common but not exclusive response (Fig. 4). This response was not as surprising, since previously we have noted that small checkerboards of high contrast and sharp edges tend to evoke mottle patterns (Barbosa et al., 2004). However, a good deal of work remains to determine experimentally the visual background features that evoke mottle.

Ontogenetic changes in disruptive patterning

Animals undergo a number of changes during ontogeny, and a particular function (e.g. behavioral, physiological, morphological, etc.) can shift as body size changes (Koehl, 2000). In most animals, ontogenetic color changes are non-reversible, and they are associated with normal progressive development of an animal (e.g. Booth, 1990). Such color changes occur in marine and terrestrial invertebrates, fish, amphibians, reptiles, birds and mammals, and are often associated with changes in size, vulnerability, reproductive status, habitat and metabolism. In cuttlefish, color changes are not fixed, and animals can change rapidly from one pattern to another. The notion of ontogenetic changes in body patterning of *Sepia officinalis* is not novel. Changes in camouflage tactics between hatchlings and adults result from a shift in emphasis from crypsis to communication (especially sexual signaling), with adults exhibiting the zebra body pattern (Hanlon and Messenger, 1988; Hanlon and Messenger, 1996). In addition, the density of chromatophores has been shown to decrease with age (Hanlon and Messenger, 1988; Hanlon and Messenger, 1996). Even though *S. officinalis* may be able to change rapidly between particular camouflaged body patterns, the combination of disruptive components shown may not be as flexible, with adult animals showing different combinations of disruptive components compared to young animals (Table 2). Furthermore, some disruptive components appear to be almost completely dropped with cuttlefish age (Fig. 7). Morphologically, the higher density of chromatophores in

hatchlings compared to adults (Hanlon and Messenger, 1988) may explain why smaller animals were more disruptive than larger animals. A higher density of chromatophores may imply that small cuttlefish are capable of expressing a given component more strongly and with greater fine tuning.

From our study, we can conclude that disruptive patterning in *S. officinalis* appears to undergo three main changes during ontogeny: (1) the degree of disruptive coloration decreases with age (Figs 5 and 6), (2) the types of components change, with some components shown more frequently in one age group, and less so in other age groups (Fig. 7), and (3) the combinations of disruptive components expressed also change with age of animal (Table 2).

Visual perception and background features

Work on young *S. officinalis* showed that when animals are placed on the same particle size, body patterning changes dramatically during ontogeny [Fig. 2 (see also Hanlon and Messenger, 1988)]. In the present study, we extended this observation to a wider range of animal sizes. For example, it is interesting to look at the body patterning response of cuttlefish of different sizes placed on a similar checkerboard size. On a 0.84 cm² checkerboard (1200% check area), hatchlings (size class 1) responded with a uniform body pattern, whereas adults (size class 6) placed on a 0.88 cm² (4% check area) responded with a mottle body pattern (Fig. 4). It is clear that for cuttlefish of different sizes, body patterns chosen on a checkerboard with a very similar area are related to the animal's size.

Our results indirectly address how cuttlefish scale their body patterns: they apparently cue visually on light objects in the visual background. Since the cuttlefish mantle has a convex shape, it appears likely that a cuttlefish cannot see its White square component. Indeed, blocking their posterior field of view by placing a plastic ruff around the head does not seem to prevent cuttlefish from showing an appropriate camouflage body pattern [see fig. 34 in Messenger (Messenger, 2001)], suggesting that cuttlefish do not require visual confirmation of their body pattern when deciding what camouflage pattern to show. It therefore seems likely that the animals do not visually inspect their skin components; i.e. their visual sensori-motor system does not rely on a feedback response.

A few other animals are also faced with the problem of what body patterns to show on a given substrate. For example, the skin of the tropical flatfish *Bothus ocellatus* is believed to be under neural control, changing within 2–8 s, but mechanisms of visual control of patterning remain undetermined (Ramachandran et al., 1996; Kelman et al., 2006). In cephalopod studies, it is clear that cuttlefish cannot adjust the size of each physiological skin component for disruptive coloration, although they can express each component in different intensities. For example, the size of the White square is fixed relative to the animal's mantle length. It continues to intrigue us that the same visual input can lead to different motor output in animals of different sizes. Unfortunately, visual processing in cephalopods is poorly studied (Messenger, 1991), thus it is impossible to provide any useful speculation about

neural mechanisms of perception ontogeny. The cuttlefish's responses on small check areas indicate that cuttlefish of all sizes possess high visual acuity. As stated by Groeger et al. in their study on the ontogenetic changes in visual acuity of *S. officinalis* (Groeger et al., 2005), at very early stages of development cuttlefish already have acute vision, although this acuity improves in larger animals.

This study is limited insofar as the experiments dealt with only one detail of the background checks: size (measured as area). In addition, it is accepted that contrast between light and dark objects in the background, as well as object edges, are essential visual cues to turn on disruptive coloration (Chiao and Hanlon, 2001a; Chiao et al., 2005; Mähger et al., 2006). According to Chiao and Hanlon (Chiao and Hanlon, 2001a) and Mähger et al. (Mähger et al., 2006), a contrast sensitivity of ca. 15–20% represents the minimum contrast level necessary to trigger the full expression of one disruptive component. In the present paper, contrast (>84%) and edge information were held constant; therefore, from this study we cannot make any inferences about their weight in determining disruptive coloration in cuttlefish, nor their role in the ontogeny of body patterning. One limitation of this work is that we used six specific check areas: 4, 12, 40, 120, 400 and 1200% of cuttlefish White square area. Previous work (Mähger et al., 2006) used 100% as the standard size that evoked disruptive coloration in *S. officinalis*, thus we are confident that 40, 100 and 120% will evoke disruptive coloration in *S. officinalis*. However, we cannot draw accurate conclusions about cuttlefish pattern responses below 40% or higher than 120%. Although the purpose of this study was not to establish all the visual features that turn on disruptive coloration, we can infer from our results that light objects in a highly contrasting visual environment with well-defined edges are an important visual cue necessary to evoke disruptive coloration in any sized cuttlefish.

Consistent with the initial research on this topic on *S. pharaonis* (Chiao and Hanlon, 2001a), *S. officinalis* responded with different body patterns to checkerboards of different sizes. However, *S. officinalis* showed disruptive patterning on a broader range of check areas than *S. pharaonis*. Both species have a wide geographical distribution. *Sepia pharaonis* is found in tropical waters, whereas *S. officinalis* can be found in a variety of temperate environments. Little is known about the ethology of either species under natural conditions, yet from our results, we speculate that there may be a larger variety of light objects in the visual backgrounds throughout the range of *S. officinalis*. However, no one has characterized visual backgrounds in any of these natural habitats with these sorts of features under consideration.

Future studies that explore the effective light areas of the background that elicit disruptive patterns may help us understand differences in visual perception and pattern scaling between large and small animals. We used a greatly simplified visual background stimulus – a high-contrast checkerboard – to decipher this ‘visual sampling rule.’ We hope this information can guide us and others to understand cuttlefish responses to natural substrates.

List of abbreviations

AHB	Anterior head bar
AMB	Anterior mantle bar
ATML	Anterior transverse mantle line
ML	mantle length
MMS	Median mantle stripes
N	number of cuttlefish
PMS	Paired mantle spots
PTML	Posterior transverse mantle line
WAT	White arm triangle
WHB	White head bar
WMB	White mantle bar
WPT	White posterior triangle
WS	White square

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