# Disruptive coloration elicited on controlled natural substrates in cuttlefish, *Sepia officinalis*

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## Summary

Cephalopods are known for their ability to change camouflage body patterns in response to changes in the visual background. Recent research has used artificial substrates such as checkerboards to investigate some specific visual cues that elicit the various camouflaged patterns in cuttlefish. In this study, we took information from experiments on artificial substrates and assembled a natural rock substrate (fixed with glue) with those features that are thought to elicit disruptive coloration in cuttlefish. The central hypothesis is that light rocks of appropriate size, substrate contrast and edge characteristics will elicit disruptive camouflage patterns in cuttlefish. By adding graded light sand in successively greater quantities to this glued rock substrate, we predicted that disruptive camouflage patterns would be replaced by progressively more uniform patterns as the visual features of rock size, contrast and edges were altered by the addition of sand. By grading the degree of disruptiveness in the animals' body patterns, we found that the results support this prediction, and that there is a strong correlation between fine details of the visual background properties and the resultant body pattern shown by the cuttlefish. Specifically, disruptive coloration was elicited (1) when one or a few light rocks of approximately the size of the animal's White square skin component were in the surrounding substrate (dark rocks alone did not elicit disruptive coloration), (2) there was moderate-to-high contrast between the light rocks and their immediate surrounds, and (3) the rock edges were well defined. Taken together, the present study provides direct evidence of several key visual features that evoke disruptive skin coloration on natural backgrounds.

## Introduction

Unlike most animals that have a fixed or slightly changeable camouflage pattern (Cott, 1940), cephalopods produce multiple camouflage patterns, and they can change them almost instantaneously, using their neurally controlled chromatophore system (Hanlon and Messenger, 1988; Hanlon and Messenger, 1996; Messenger, 2001). The expression of camouflage body patterns is a visually driven behavior, and in laboratory experiments it has been shown that certain background variables, such as contrast, brightness, edge and size of objects, etc., are crucial for the expression of these patterns (Holmes, 1940; 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; Shohet et al., 2006; Barbosa et al., 2007; Kelman et al., 2007).

Although there is a good deal of variation in the details of the body patterns shown by cuttlefish for camouflage, the variations fall under three pattern types: (1) uniform (or uniformly stippled), (2) mottle and (3) disruptive (Hanlon and Messenger, 1988; Hanlon, 2007). The overall framework that guides our

experimental testing of this visual sensorimotor system is that cuttlefish (and cephalopods in general) might be using a simple visual cue (or a 'sampling rule') for each of the three primary camouflage pattern types. Uniform backgrounds elicit uniform body patterns in cuttlefish (Hanlon and Messenger, 1988; Chiao and Hanlon, 2001a; Mäthger et al., 2006) (Fig. 1A,B). Yet perfectly uniform backgrounds in nature are rare. On non-uniform backgrounds, disruptive camouflage can be evoked on a checkerboard in which the light squares are roughly equal in area to the White square (WS) skin component shown on the mantle of cuttlefish (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b) (Fig. 1C). Furthermore, large numbers of small black and white checkers with areas of roughly 4 and 12% of the animal's WS component (see Fig. 1E for location of WS) elicit mottled body patterns (Barbosa et al., 2004). We have subsequently been able to elicit mottle patterns on natural substrates in the laboratory (A.B., L.M.M. and R.T.H., unpublished results).

Cuttlefish are not expected to match artificial backgrounds, such as checkerboards, but rather to use the simple and few
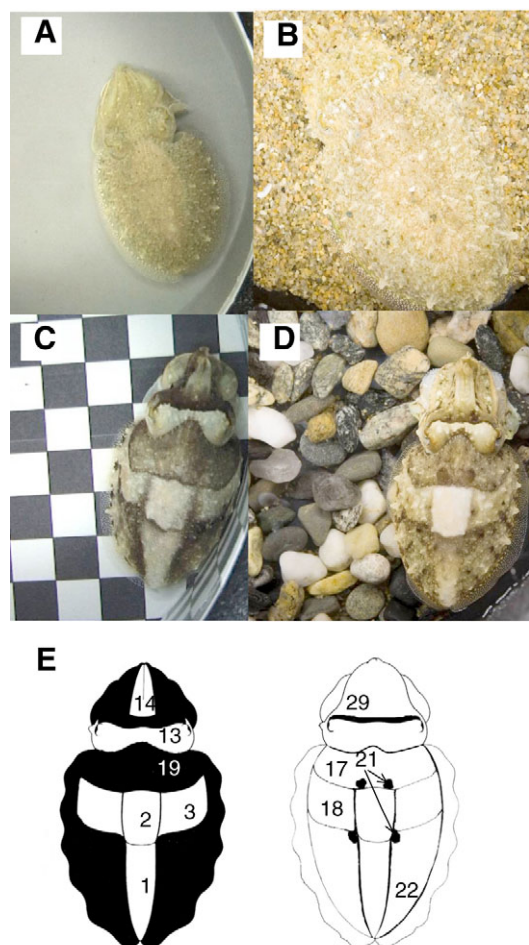
Fig. 1. Uniform and disruptive body patterns on artificial and natural backgrounds. (A) Uniform body pattern on a uniform artificial gray background and (B) uniform sand. (C) Cuttlefish showing disruptive coloration on a black and white checkerboard for which the white checks are roughly equal in size to the animal's White square component (component 2 in Fig. 1E). (D) Disruptive coloration on natural substrate with contrasting dark and light rocks. (E) Disruptive components that were graded (see text for detail on grading method). Light chromatic components: 1, White posterior triangle; 2, White square; 3, White mantle bar; 13, White head bar; 14, White arm triangle. Dark chromatic components: 17, Anterior transverse mantle line; 18, Posterior transverse mantle line; 19, Anterior mantle bar; 21, Paired mantle spots; 22, Median mantle stripe; 29, Anterior head bar. Components were originally described and numbered by Hanlon and Messenger (Hanlon and Messenger, 1988). For consistency, we have listed these numbers here.

visual cues to attempt camouflage. This robust behavioral assay has subsequently been used to show that cuttlefish cue visually on area, not the shape or aspect ratio, of light objects on a dark background and that substrate edges and contrast are important in eliciting specific body patterns (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005; Mäthger et al., 2006; Barbosa et al., 2007).

On a background of natural materials, cuttlefish show disruptive coloration when settled on a mixture of light and dark objects (Fig. 1D). This was first shown by Holmes (Holmes, 1940). The patterns that make up disruptive

coloration are thought to break up the recognizable outline of the animal by creating 'false' lines and edges (Cott, 1940), and it has been shown experimentally that disruptive coloration is a successful visual mechanism to achieve camouflage, at least against bird predators (Cuthill et al., 2005; Stevens et al., 2006).

Although it is well known that cuttlefish are masters of disguise, no controlled studies on natural substrates have yet been performed. Cuttlefish are known to show disruptive coloration on natural gravel as well as a picture of natural gravel (Chiao et al., 2005). However, the visual features of the substrate were manipulated by changing the contrast and edge features of the two-dimensional picture of the substrate, not the natural substrate itself. In the present study we expanded this idea. We took information (e.g. object size, contrast, edge, etc.) from many recent experiments on artificial substrates and created a substrate using natural rocks glued permanently to a Plexiglass sheet. This substrate's features (e.g. the spectral reflectance of rocks, contrast, edges) were those thought to elicit disruptive coloration in cuttlefish. We then added graded light sand in successively greater quantities to this glued rock substrate, thus altering the substrate's visual features.

The aim of this study was to examine the visual cues that are required to elicit disruptive coloration in a controlled and measured natural scene. The results confirm previous laboratory studies using artificial substrates but, more importantly, they extend our understanding of the visual sampling rules that govern cuttlefish camouflage behavior in nature.

## Materials and methods
### Animals

Nine cuttlefish, *Sepia officinalis* (Linnaeus 1758), ranging in size from 9–11 cm mantle length (ML), were used in this study. All cuttlefish were raised from eggs at the Marine Resources Center (MRC) of the Marine Biological Laboratory (Woods Hole, MA, USA).

### Substrates

Various rocks were collected at a local beach in Woods Hole and glued onto a Plexiglas sheet using aquarium sealant. The resulting substrate was a circular area covered by rocks (34 cm in diameter; S1 in Fig. 2). It contained several visual cues known to evoke disruptive patterning in cuttlefish: (1) light objects roughly equal in area to that of the cuttlefish WS component, (2) high contrast and (3) distinct edges (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005). Substrates are referred to as S1–S6 (Fig. 2). Substrate S1 was used in all consecutive trials, in which sand was added to S1 to gradually remove these disruptive cues (S2–S6 in Fig. 2). Sand was collected locally and dried before being sieved at the Sedimentation Laboratory of the United States Geological Survey (Coastal and Marine Geology Program, Woods Hole Science Center, MA, USA). We chose mean grain size $\phi$ 0 (1 mm diameter) for this experiment [$\phi=-\log_2$ (diameter in mm)]. The experiment consisted of six experimental treatments: (1) the rock substrate without any sand, S1; (2) rock substrate with half a cup of $\phi$ 0 sand spread evenly over the substrate, S2 (one cup=0.2366 l); (3) one cup of sand, S3; (4)

two cups of sand, S4; (5) four cups of sand, S5; (6) sand only, S6. Substrate 6 consisted of φ 0 sand glued onto a Plexiglas sheet, and covered with three cups of loose sand. There were no rocks in substrate 6. The effects of increasing the amount of sand on the rock substrate were to reduce the number of light

rocks in the visual environment, as well as to decrease overall contrast and alter edge characteristics. Cuttlefish were exposed to trials in random order.

### Experimental set-up

Trials were performed in an experimental tent covered with black plastic sheeting, preventing visual distraction to the animals. The substrate was placed in a small tank (55 cm×39 cm×13 cm) supplied with running sea water. A circular plastic arena (24 cm diameter, 10 cm height), lined with black felt, was placed over the substrate. A Sony DCR-VX1000 digital video camera was mounted above the tank, and controlled remotely. A monitor outside the chamber allowed observation during the trial without disturbing the animal. Lighting was provided by a circular fluorescent light (Sylvania circline daylight, 40 W; Osram Sylvania, Munich, Germany; light intensity at the surface of the substrate was approximately 1000 Lux).

### Procedure

Every animal was allowed a minimum of 5 min acclimation time. An animal was considered acclimated when excessive hovering movements had ceased and a stable body pattern was shown. The camera was set to record for 1 s every 30 s. Trials were 30 min long, yielding 60 s of total footage. Sand (S2–S5) was removed from the substrate using a siphon.

### Disruptive grading

From the video recordings, we took 10 images per animal per substrate, yielding a total of 540 images that were graded for disruptiveness using the grading scheme described in Mäthger et al. (Mäthger et al., 2006). To prevent experimenter bias, images were randomly renamed before grading, and the background was removed using Adobe Photoshop. The origin of images was only re-established after grading was completed. Disruptive patterning in *Sepia officinalis* most commonly consists of 11 individual dark and light components, which are independent physiological units that can be shown singly or in combination with each other (Hanlon and Messenger, 1988). The components are produced by selective expansion (for dark components) and retraction (for light components) of chromatophores, which either expose or cover underlying white reflectors (see Fig. 1E for a description of which chromatic components were used for grading). After studying many thousands of images, we have written a detailed handbook for laboratory use, describing each component's level of expression and outlining how to grade each component. Each component can be shown with varying degrees of expression, from 0 (no expression) to 1 (weak expression), 2 (moderate expression) and 3 (strong expression). Using this grading scheme, an animal can
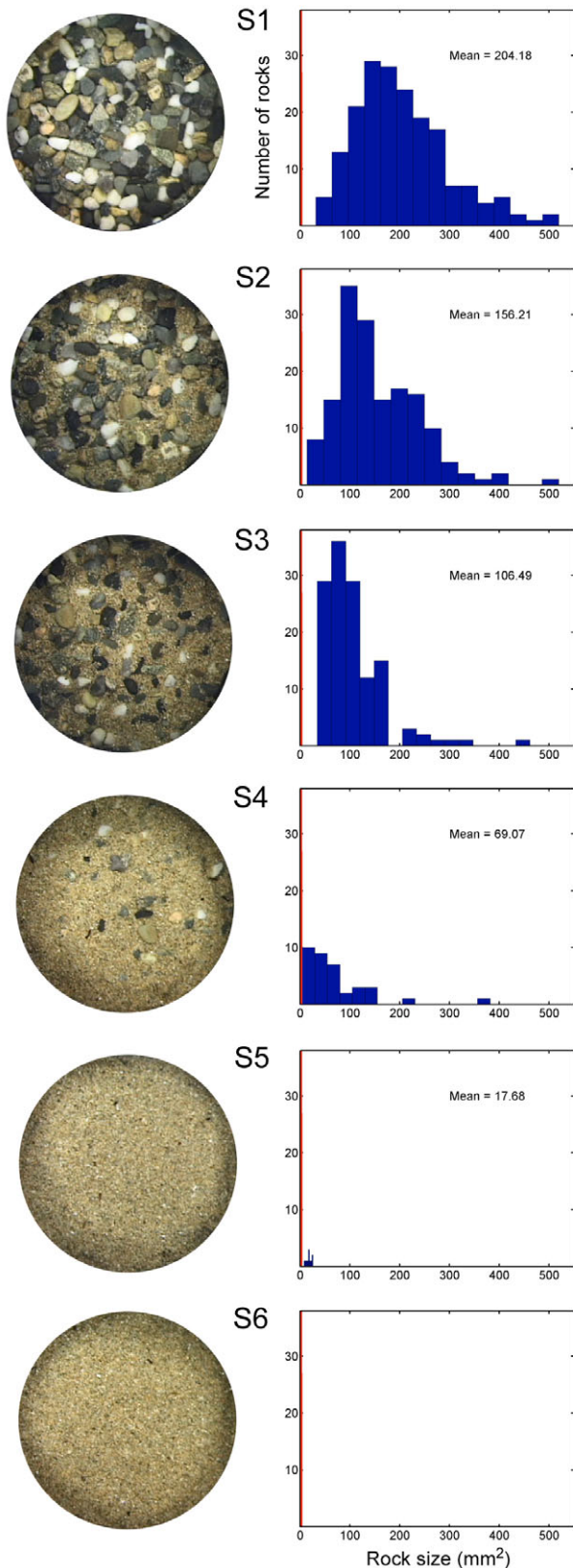


Fig. 2. Spatial properties of six natural substrates (S1–S6) used in this study. Left column, images of substrates; right column, rock size distributions. Rock size scales (i.e. *x*-axis) of plots are kept the same to show shift towards the left (i.e. smaller size) of mean rock size. Red vertical line on the very left of each plot is truncated sand grain size. S1, rock substrate without any sand; S2, rock substrate with half a cup of the φ 0 sand spread over the substrate evenly; S3, one cup of sand; S4, two cups of sand; S5, four cups of sand; S6, full sand.

be given a total grade ranging from 0 (no expression of any disruptive components) to 33 (maximum expression of all 11 disruptive components, resulting in a strongly disruptive body pattern).

### Characterization of substrate features

*Image analysis*

Still images of the six substrates (S1–S6 in Fig. 2) were taken using a Sony DCR-VX1000 digital video camera. These images were used to characterize several substrate features, including rock size distribution, sand coverage, overall contrast and number of light objects in the scene. Because the settings of the camera were automatically adjusted when these images were taken, it was impossible to derive overall intensities of all six substrates directly from these still images. Instead, spectral reflectance measurements of rocks and sand were used to calculate relative mean intensities (see below). To determine the rock size distribution of each substrate, rock areas were individually measured using an image processing and analysis program (US National Institute of Health; http://rsb.info.nih.gov/nih-image/). The WS areas of the nine cuttlefish were also measured to assess the relationship between average rock size and average WS size. The sand coverage for each substrate was computed directly by subtracting visible rock areas from the total area. For characterizing global contrasts of natural scenes, we computed Root-Mean-Square (RMS) contrast ($C_{rms}=\sqrt{\{[\Sigma I^2-(\Sigma I)^2/N]/N\}}$, where $I$ is individual pixel values and $N$ is the total number of pixels). The $C_{rms}$ is commonly used to gauge the overall contrast of natural images (e.g. Bex and Makous, 2002). Pixel values in each image were used to calculate RMS contrast directly. In addition, the number of light objects in each substrate was determined by computing Weber contrast [$WC=(I_{object}-I_{background})/I_{background}$, where $I_{object}$ is the intensity of each rock and $I_{background}$ is the averaged intensity of the entire image] and then counting the numbers of rocks whose Weber contrasts were greater than 1. Typically, any object with a Weber contrast greater than 1 means that its intensity is at least twice that of the background. Similar to vertebrate vision (e.g. Shapley and Enroth-Cugell, 1984), the cuttlefish visual system may use an early transformation of the visual input akin to the Weber contrast transformation. The divisive normalization by substrate mean intensity embodied by this transformation amplifies the salience of light objects on a background. Although counting the numbers of rocks whose Weber contrasts were greater than 1 is arbitrary in nature, it provides a meaningful measure of object salience on a given substrate.

To examine the edge characteristics of S1–S6, and evaluate whether cuttlefish can be discerned on a given substrate by the edge-detection mechanisms of potential predators, a standard Laplacian of Gaussian (LoG) operator was applied to images of substrates with and without an animal. The sensitivity threshold for the LoG operator was set the same across all images, and the standard deviation (the $\sigma$ value) of the LoG operator was 2. In a supplementary figure (supplementary material Fig. S1) we present additional results with $\sigma$ values of 1 and 3.

The LoG operator was first proposed to implement an edge-detection algorithm that is similar to the receptive field property of retinal ganglion cells in the vertebrate retina (Marr and Hildreth, 1980). In brief, edges (defined as abrupt changes in intensity within an image) can be located by taking the first derivative ($\delta I/\delta x$ and $\delta I/\delta y$) of an image $I(x,y)$ in both $x$ and $y$ dimensions. However, at a gradient peak in the first derivative, by taking the second derivative ($\delta^2 I/\delta x^2$ and $\delta^2 I/\delta y^2$), the presence of 'zero-crossings' can be depicted, which represent the positions of edges in an image. Performing a derivative twice in succession is equivalent to the Laplacian operator [$\nabla^2=\delta^2 I/\delta x^2+\delta^2 I/\delta y^2$]. Because of noises in the processed image, a Gaussian operator {$G(x,y)=exp[-(x^2+y^2)/(2\pi\sigma^2)]$}, where $\sigma$ is the standard deviation of the Gaussian function} is often required to smooth the image before applying the Laplacian operator. These two operations can be combined effectively to create the LoG operator [$\nabla^2 G(x,y)=\delta^2 G/\delta x^2+\delta^2 G/\delta y^2$].

The LoG operator has been suggested as a biologically plausible detector algorithm, although other efficient algorithms are available (Marr and Hildreth, 1980; Stevens and Cuthill, 2006). Edge detection plays an important role in differentiating between an object and its background, and the vertebrate visual system has been shown to have edge-detection mechanisms that may aid in object recognition (Burr et al., 1989; Gordon, 1997).

*Spectral measurements*

Using a spectrometer (USB2000; Ocean Optics, FL, USA), reflectance spectra were taken of 83 rocks of the substrate. We used the same circular light source as that used during experiments. This provided an even and diffuse field of illumination. The fiber (positioned vertically) was set to measure an area with a diameter of 2 mm. Each rock was measured two to three times and measurements were averaged. A diffuse reflection standard (WS-1, Ocean Optics) was used to standardize measurements. Eighteen reflectance spectra were taken of the sand, and these were averaged to yield a single reflectance value.

After measuring the reflectance spectra of the rocks and sand used in this study, the relative photon catch (PC; amount of light absorbed by a photoreceptor and available for vision) was determined. This is given by: $PC=\int[(1-exp(-kS(\lambda)l)\times R(\lambda)]d\lambda$, after Warrant and Locket (Warrant and Locket, 2004), where $S(\lambda)$ is the spectral sensitivity of the visual pigment, $R(\lambda)$ is the spectral composition of the light reflected from the rock/sand, $l$ is the length of the rhabdom [400 μm (Hanlon and Messenger, 1996)] and $k$ is the quantum efficiency of transduction [0.0067 μm$^{-1}$ (Warrant and Nilsson, 1998); for further details, see Mäthger et al. (Mäthger et al., 2006)].

To compute the relative mean intensity, we calculated the weighted sums of $PC_{rock}$ and $PC_{sand}$ for each substrate using sand coverage as a weighting factor, and normalized the PC relative to a white surface. Weber contrasts of rocks derived directly from spectral measurements were also calculated [$WC=(PC_{object}-PC_{average})/PC_{average}$, where $PC_{object}$ is the PC of the rock under consideration and $PC_{average}$ is the averaged PC of all reflectance spectra]. This allowed us to compare this method with the previous image analysis method.

### Consideration of light versus dark objects in the background

Previous experiments performed on artificial substrates showed that an important background feature eliciting

disruptive coloration is the presence of light objects on a dark background [not dark objects on a light background (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b)]. To test this on natural substrates, we looked in detail at the cuttlefish images obtained on substrate 4 (two cups of sand). We divided all images (10 images per animal, total of 90) into 'disruptive' and 'non-disruptive' and counted the number of light and dark rocks in the vicinity of the animal ('vicinity' was defined as a circular region of interest with a radius of 1 ML around the center of the animal's head). Only rocks of sizes ranging from 40–120% of the animal's WS area were counted. On artificial substrates, we learnt that the sizes of light objects need to be approximately 40–120% of the WS area in order to elicit disruptive coloration (e.g. Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Barbosa et al., 2007). Using the spectral reflectance data from the substrate rocks and sand, we computed Michelson contrast [$MC = (PC_{rock} - PC_{sand})/(PC_{rock} + PC_{sand})$, where $PC_{rock}$ is the PC of each rock and $PC_{sand}$ is the PC of the sand; only absolute values are used] between the sand and each rock. Because Weber contrast is used in a more global context, using Michelson contrast was preferable because it is generally used for side-by-side regular repeating patterns. The rocks included in this count ranged in contrast from 29–77% (dark rocks) and 39–78% (light rocks).

Because of the small sample size of this part of the analysis, we did not perform any statistical tests. Instead, in a separate experiment, we presented cuttlefish with four additional substrates: (A) pure sand (grain size φ 0), (B) sand with 15 black rocks spread evenly, (C) sand with 15 white rocks spread evenly throughout the arena, and (D) sand with eight white and eight black rocks distributed evenly. Substrates are referred to as SA to SD. The rocks ranged in size from 0.2 cm$^2$ to 0.7 cm$^2$; which equaled approximately 35–100% of the animals' WS component. The Michelson contrast (see above for method) between the rocks and sand was high: 75% (sand *versus* white rocks) and 73% (sand *versus* black rocks). Twelve cuttlefish, ranging in size from 3.0 to 3.5 cm ML, were used.

### Results

#### *Substrate features*

Because each substrate was reassembled every time a trial was set up (i.e. sand added to the glued rock substrate), the spatial features varied somewhat between trials. Also, an animal's movements during a trial occasionally changed the visual features (because of slight sand blowing). However, the overall visual effect of increasing the amount of sand added to the substrate was similar for all trials. As the images in Fig. 2 illustrate, increasing the amount of sand covering the rock substrate resulted in fewer rocks being visible. Measurements of rock sizes showed that adding sand to the substrate apparently reduced the size of the rocks to an observer by covering more and more rock area (plots in Fig. 2). Previously, we have shown that cuttlefish produce strong disruptive body patterning on artificial backgrounds when the size of light objects on a dark surrounding is roughly 40–100% of the animal's WS skin component (Chiao and Hanlon, 2001a; Mäthger et al., 2006; Barbosa et al., 2007). Thus, we felt it was interesting to compare the average rock size in S1–S6 with the average WS size of nine cuttlefish placed on these natural substrates. Overall, the

average rock size was 54% of WS size on S1, and it gradually decreased to 41%, 28%, 18% and 5% of WS size on S2–S5, respectively (Fig. 3A).
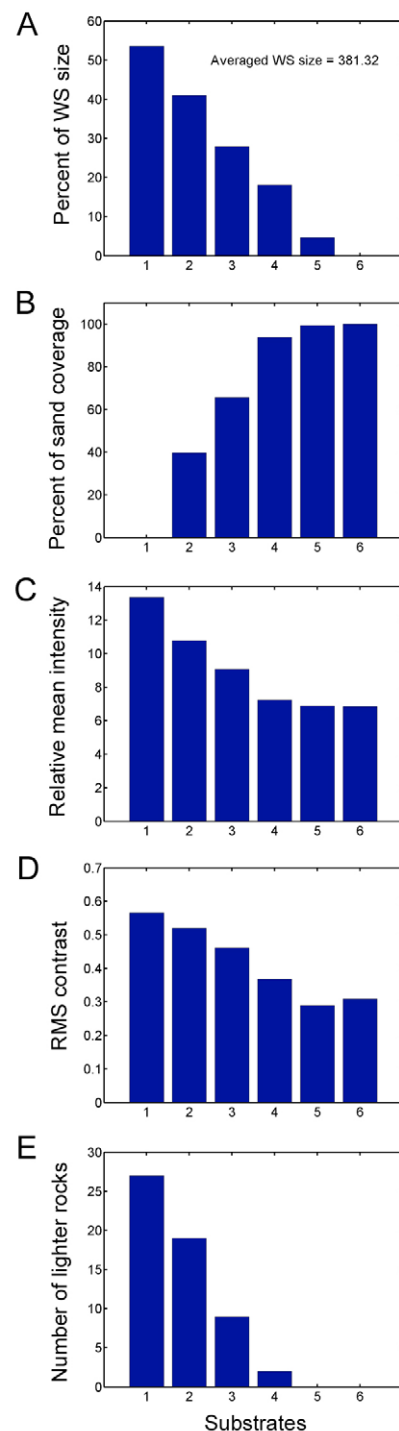


Fig. 3. (A) Mean rock size of S1–S6 as percentage of nine cuttlefish's average White square area (381.32 mm$^2$). (B) Percentage of sand coverage of S1–S6. (C) Relative mean intensity of S1–S6. Unit represents percentage of average photon catch of each substrate relative to photon catch of a white surface (see text for photon catch calculation). (D) Root-mean-square (RMS) contrasts of S1–S6. (E) Number of lighter rocks of S1–S6. Lighter rocks are defined by Weber contrasts greater than 1 (see text for the Weber contrast calculation).

The rate of sand coverage (expressed as percent of substrate covered) can be seen in Fig. 3B. Although no sand was used on S1 (rocks only), sand was added on S2 to S6, reaching 100% coverage for S6. Note that four cups of sand covered most of the rocks, and thus the sand coverage was close to 100% in S5. Relative mean intensities derived from reflectance measurements indicate that all six substrates are low in brightness (compared with a white surface), and averaged intensities slightly decreased from S1 to S6 (Fig. 3C). In Fig. 3D, we show that the global contrast (expressed as RMS contrast) is highest in S1 and drops to low contrast in S5 and S6.

The number of lighter rocks visible in each substrate, shown in Fig. 3E, illustrates specifically that, as sand is added to the rock substrate, fewer and fewer white rocks are visible. As expected, this correlates with a reduction in the animals' disruptive coloration (see below).

Fig. 4 shows the Weber contrast for three white rocks in substrate S1 (Fig. 4A, circled in color; reflectance spectra shown in Fig. 4B). In comparison with the average reflectance spectra of the substrate (dark line in Fig. 4B), the Weber contrast of the three white rocks was 2.8, 1.87 and 1.81. Note that the intensities of the three white rocks are saturated in image S1 of Fig. 4A (pixel values are 255 for all three rocks); however, spectral reflectance measurements reveal that the rock outlined in blue is more reflective than the other two rocks. In Fig. 4C, we show the Weber contrast distribution of all rocks of S1. The plot indicates that most of the rocks are either darker (negative values) or lighter (positive values) than the averaged background. In total, 13 rocks had a Weber contrast greater than 1.

Note also the difference in results depending on methodology. Fig. 3E (based on image intensities) shows that 27 rocks had a Weber contrast greater than 1, whereas in Fig. 4C (based on spectral reflectance measurements) only 13 rocks had a Weber contrast greater than 1. This indicates that the intensity-based characterization in Fig. 3 may slightly overestimate Weber contrast (because of the non-linearity and limited dynamic range of the camera), whereas the spectral reflectance-based calculation in Fig. 4 captured individual Weber contrast more reliably.

### Body pattern changes

The body patterning shown by cuttlefish changed in response to increasing the amount of sand to the rock substrate (Fig. 5A). Although animals showed strong disruptive patterning when on S1 (no sand), the disruptive grade decreased as sand was added. Animals did not show any disruptive coloration on S5 (four cups of sand) and S6 (full sand).

### The influence of light and dark rocks on disruptive coloration

The spatial features of S4 (two cups of sand) and the corresponding body patterns observed varied between trials, with animals showing both disruptive and non-disruptive coloration. We looked in detail at the images obtained on S4 and counted the number of dark and light rocks in the vicinity of the animal. In Fig. 5B we show that the presence of dark rocks did not have any bearing on disruptive body patterning. However, disruptive body patterning was shown on substrates that had a larger number of white rocks. There were comparable numbers of dark rocks in the disruptive and non-disruptive group (average of 4.3 *versus* 3.5 rocks, respectively; ratio of 1.2:1). By contrast, the number of light rocks differed between the disruptive and non-disruptive group (average of 1.2 *versus* 0.3 rocks, respectively; ratio of 4.6:1).

In a follow-up experiment, we presented cuttlefish with four substrates (Fig. 5C): (A) pure sand, (B) sand with black rocks spread evenly, (C) sand with white rocks spread evenly, and (D) sand with white and black rocks spread evenly. Cuttlefish showed non-disruptive body patterning when on SA (sand) and SB (sand with black rocks); the body patterns shown on these substrates did not differ statistically ($t=1.39$, $P=0.19$, $N=12$). Disruptive patterning was observed only on SC (sand with white rocks) and SD (sand with white and black rocks; Fig. 5C). The body patterns shown on SC and SD differed significantly from those shown on sand ($t=8.7$, $P<0.001$, $N=12$ for SC; $t=6.33$, $P<0.001$, $N=12$ for SD).

### Edge characteristics

Adding sand to the rock substrate S1 modified edge characteristics of the rocks. Fig. 6 shows that the edges become less conspicuous and increasingly fragmented as more sand is added to the substrate. This correlates with the apparent reduction in the number and size of the rocks (Fig. 2). The different body patterns shown on S1–S6 may thus also correlate with edge information of each substrate. By applying a standard edge-detection algorithm (LoG), we show that creating false edges in disruptive patterns makes cuttlefish less apparent in
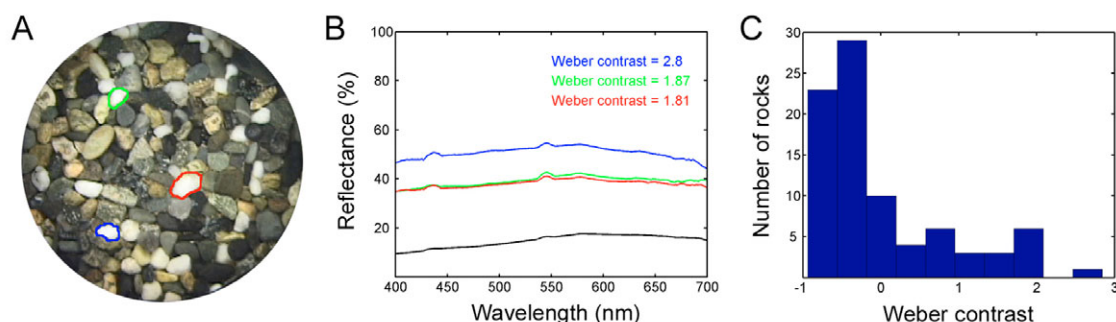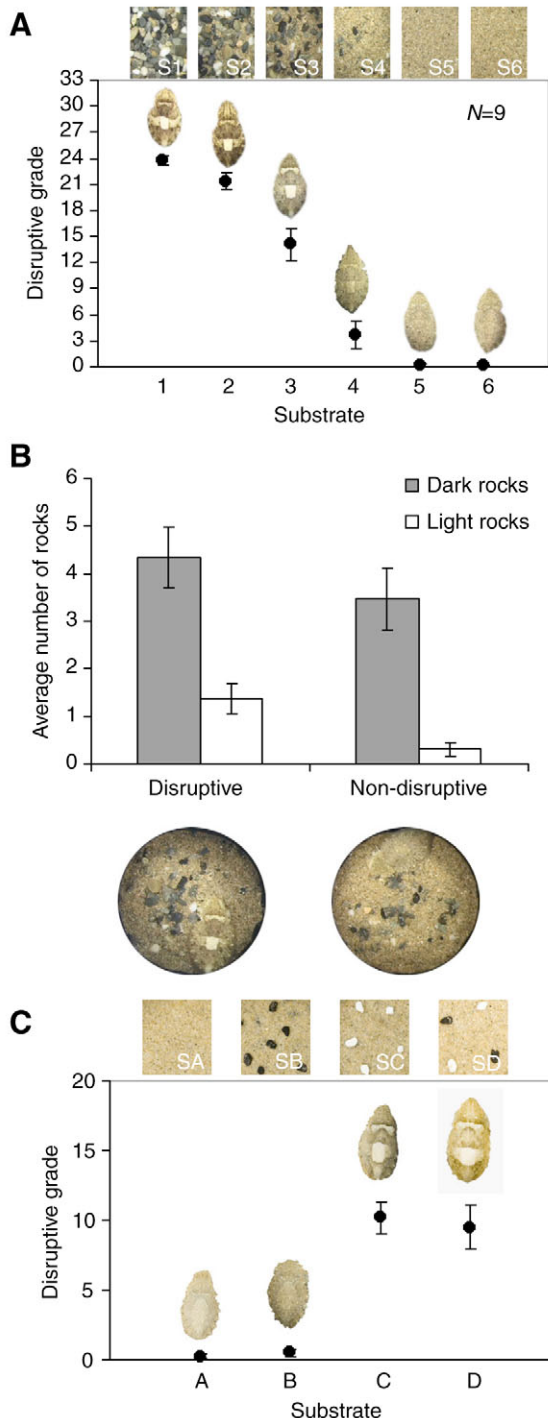


Fig. 4. Spectral properties and Weber contrast of selected rocks of S1. (A) Substrate S1 showing outline of three light rocks. (B) Reflectance spectra of three rocks marked in A, and the average reflectance spectrum of all rocks (black). (C) Weber contrast distribution of all rocks of S1.

these LoG-filtered images (Fig. 6, compare the second and third columns). By contrast, covering the substrate with sand reduces edge information in the scene, and cuttlefish with uniform patterns are just as difficult to detect using a LoG operator (Fig. 6E,F) because uniform body patterns do not have false edges, as those seen in disruptive coloration. Although these LoG-filtered images were generated using only one $\sigma$ value (representing one receptive field size), other $\sigma$ values (corresponding to different receptive field sizes) were also implemented (see supplementary material Fig. S1), and the general trend holds true across all different $\sigma$ values.



Fig. 5. (A) Average grade of cuttlefish disruptive body patterning shown on substrates S1–S6. Cuttlefish were highly disruptive on S1 and their disruptive scores diminished as more sand was added to the substrate. Grading scores were low for S5 and S6. Images are representative body pattern shown on each substrate. Error bars are s.e.m.; $N$=9. (B) Number of dark and light rocks in animals' vicinity as a function of body patterning (disruptive and non-disruptive; see text for details). In the presence of dark rocks, animals showed disruptive or non-disruptive coloration, depending on whether or not light rocks were present. Light rocks consistently evoked disruptive coloration. Images are examples of body patterns shown. Data obtained from trial on S4 (total of 90 images). Error bars are s.e.m. (C) Average grade of disruptive body patterning shown on four substrates (SA–SD). Disruptive coloration was shown on sand with white rocks and sand with white and black rocks (SC and SD). Animals were non-disruptive on sand and sand with black rocks (SA and SB). Error bars are s.e.m.; $N$=12.

## Discussion

Cuttlefish color change has been the subject of several studies of camouflage (Holmes, 1940; Hanlon and Messenger, 1988; Hanlon and Messenger, 1996; Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005; Shohet et al., 2006; Kelman et al., 2007). Even though it is known that cuttlefish are masters of disguise both in the laboratory and in nature, there are no quantitative accounts of cuttlefish camouflage on natural substrates.

Most of our knowledge on cuttlefish camouflage comes from laboratory studies using artificial substrates, such as checkerboards and similar two-dimensional patterns. The advantage of using checkerboard substrates is that specific variables can be changed one at a time, allowing for well-controlled experiments (e.g. Ramachandran et al., 1996; Chiao and Hanlon, 2001a; Mäthger et al., 2006). By contrast, natural substrates are highly variable in size, contrast, brightness, color and texture, and this makes experimentation with them challenging.

### *Object size, contrast and edge determine expression of disruptive coloration in cuttlefish*

In our study, we kept the baseline rock substrate steady by gluing rocks in place. The visual features were then altered by adding sand in known quantities. Even though every substrate differed slightly from trial to trial because of the animals' movements, causing sand to be slightly shifted, the characteristics of each substrate (e.g. numbers of light and dark rocks visible, contrast, edges, etc.) changed in the same manner.

Adding sand to the rock substrate had several visual effects: (1) it visually decreased the size, number and spatial distribution of rocks, (2) it filled in the spaces and eliminated sharp edges that were created by shadows cast between rocks, and (3) it fragmented and reduced the number of long conspicuous edges. As fewer light objects were visible and the visual environment became increasingly uniform, cuttlefish responded correspondingly by reducing their disruptiveness and becoming more uniform. Furthermore, fewer and fewer rock edges were visible and, correspondingly, the disruptive pattern lost more of its false edges, particularly in the skin components Transverse mantle lines, Anterior head bar and Median mantle stripes (Fig. 6; see Fig. 1E for disruptive components). These

disruptive components are effective in breaking body outlines (Cott, 1940; Hanlon and Messenger, 1988; Cuthill et al., 2005; Stevens and Cuthill, 2006; Stevens et al., 2006), and help render both false and true edges of animals detectable but not recognizable on a rock substrate characterized by distinct edges. When no edges were visible in the background, the animals became uniform and minimized detection of their true body outline by burying in the sand. That is, in the parlance of Cott

(Cott, 1940), they switched from disruptive coloration to general resemblance of the background. In our edge analysis, we limited ourselves to only one edge-detection algorithm (LoG, $\sigma$ values 1, 2, 3; Figs 6, 7, supplementary material Fig. S1). There are numerous edge-detection algorithms available and many of those would without doubt detect edges of even the most camouflaged cuttlefish. However, this was not the purpose of this study, and for illustrative purposes, the LoG, which has been suggested as a useful biological edge-detection algorithm (Stevens and Cuthill, 2006), appeared as an effective operator. It is interesting to compare the camouflaged patterns analyzed in Fig. 6 with those of Fig. 7, in which we show an image of a cuttlefish that chose disruptive coloration on natural sand. The animal is easily visible, but in addition, the LoG operator tells us that the animal's false lines (around the WS and Head bar) stand out conspicuously, making it vulnerable to potential predators that use edge information to locate prey.

From these and other experiments (e.g. Hanlon and Messenger, 1988; Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005; Mäthger et al., 2006; Barbosa et al., 2007; Kelman et al., 2007), we can conclude that disruptive coloration is the preferred camouflage pattern of cuttlefish under the following known circumstances: first, when the environment contains a few light objects with sizes comparable to the area of the WS component within a visual field of approximately 1 ML around the animal. Note that dark objects alone on a background, even with contrasts similar to light objects, definitely do not evoke disruptive coloration in *S. officinalis*. Second, when the contrast levels of light objects *versus* background are high (Michelson contrast of between 40 and 80%). Third, when the visual environment is characterized by distinct edges around large objects. However, Chiao et al. showed that merely having object edges without contrast between the objects is not enough to evoke disruptive coloration (Chiao et al., 2005).

Interestingly, very little mottling was evoked by any of the six substrates (S1–S6). Previous experiments have shown that mottled patterns are evoked on non-uniform substrates with a large number of high-contrast small black and white checks with areas of roughly 4 and 12% of the animal's WS components (Barbosa et al., 2004; Barbosa et al., 2007). This indicates that background features including object size, contrast and edge information were such that either disruptive or uniform patterns were the most appropriate body
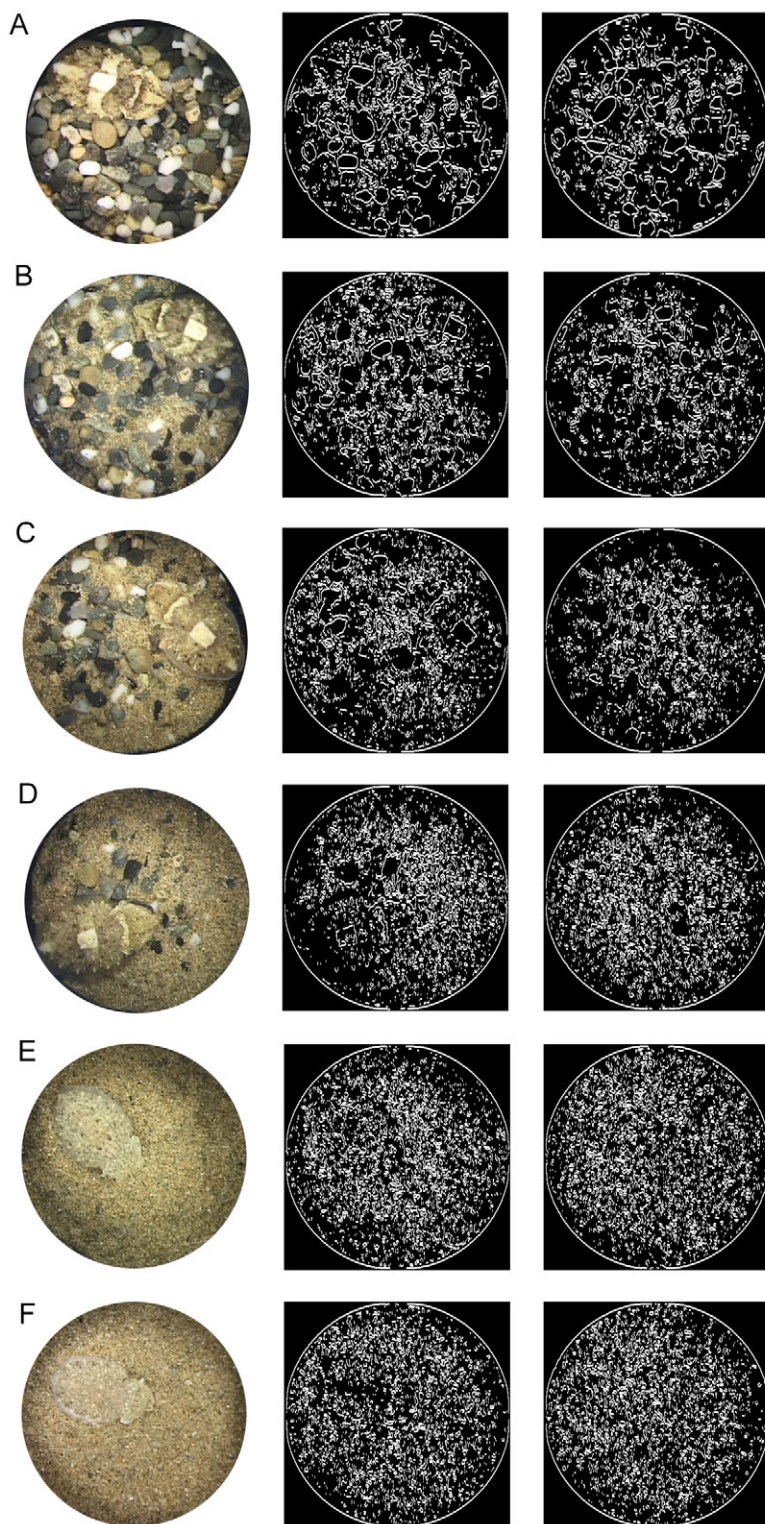


Fig. 6. Cuttlefish increase number of false edges or reduce visibility of true edges in response to different spatial properties of the substrate. (A–F) First column: original images of animals on substrates S1–S6. Second column: filtered images using edge-detection algorithm, Laplacian of Gaussian (LoG). Third column: the same LoG-filtered images of substrates only (i.e. S1–S6 in Fig. 2). Cuttlefish are difficult to detect, even with LoG operator.
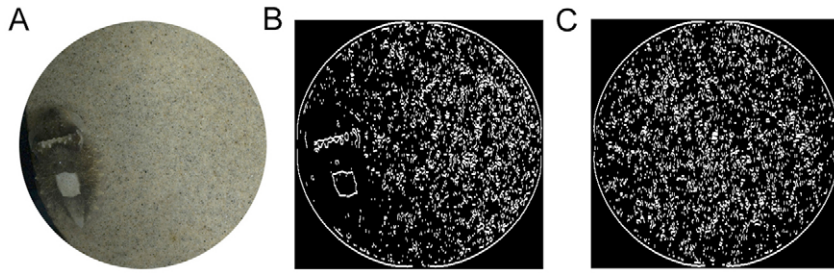
Fig. 7. (A) Image of cuttlefish showing disruptive coloration on sand (i.e. no camouflage). (B) Filtered image using same LoG edge-detection algorithm as in Fig. 6. (C) LoG-filtered image of substrate only. This figure demonstrates that in addition to a human observer being able to discern the animal on the sand substrate easily, the LoG shows that the animal's false edges stand out conspicuously. This makes parts of the animal's body distinct objects on sand.

pattern to show, and that we need to vary some or all of these features if we wish to evoke mottled body patterns.

### *Chromatic skin components limit the type of background objects used as visual cues*

In disruptive coloration, an animal's appearance is broken into several large and high-contrast dark and light areas that function partly by taking an observer's attention away from the true body outline of the animal (Cott, 1940). Depending on the environment of the animal, the light areas of the animal's pattern can be a variety of shades; however, in many animals, they are bright white (e.g. birds, fish, snakes, sharks, insects, crustaceans), which is an indication that white plays an important role in animal camouflage (Cott, 1940; Graul, 1973; Edmunds, 1974; Merilaita, 1998; Myrberg, 1990; Peterson and Peterson, 2002).

Disruptive coloration in *S. officinalis* is typically made up of 11 distinct chromatic skin components, five light components (when fully expressed, these are bright white in white light) and six dark components (when fully expressed, these are dark brown) (Hanlon and Messenger, 1988) (see Fig. 1). These components are distinct neurophysiological units made up of thousands of chromatophores that are innervated directly from the brain (Hanlon and Messenger, 1996; Messenger, 2001; Tublitz et al., 2006). Although cuttlefish can change patterns quickly, this means that they are limited in diversity by the fixed number of light and dark chromatic components they can show, and consequently, this will affect which visual cues turn on a particular camouflage pattern.

This and previous studies have shown that cuttlefish cue on well-defined large light objects in their environment to turn on disruptive coloration and that the object size must be comparable to the animal's WS component (Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b; Chiao et al., 2005; Barbosa et al., 2007). The WS component is one example that illustrates how disruptive coloration may work by creating an illusion so that an observer perceives the conspicuous WS as a random sample of the background (e.g. light rock), rather than the back of a cuttlefish. It is interesting to note that, with some exceptions, the light components (e.g. WS, White head bar, White anterior triangle) generally comprise a larger area of the skin compared with the dark components (e.g. Median mantle stripes, Anterior and Posterior transverse mantle lines), which may partially explain why cuttlefish cue on large light but not dark objects.

In summary, cuttlefish prove to be ideal organisms for the study of camouflage because within a fraction of a second they analyze complex visual fields and translate the newly acquired information into the most appropriate body pattern (Hanlon and Messenger, 1996; Marshall and Messenger, 1996; Chiao and Hanlon, 2001a; Chiao and Hanlon, 2001b). In this study we examined the visual cues required to produce disruptive camouflage in cuttlefish, *S. officinalis*, placed on one particular type of natural substrate. By continually testing our ideas of how cuttlefish perceive complex backgrounds, we hope eventually to understand the specific visual stimuli that evoke specific types of camouflage patterns. Before we can fully understand the rules that govern disruptive coloration, specific laboratory experimentation with quantitatively controlled visual stimuli combined with tests of increasingly natural materials (which have three-dimensionality that computer-generated substrates lack) must continue in parallel with field studies, which have only rarely been attempted.

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