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Author for correspondence:
Roger T. Hanlon
e-mail: rhanlon@mbl.edu

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Dynamic masquerade with morphing three-dimensional skin in cuttlefish

Deanna Panetta, Kendra Buresch and Roger T. Hanlon

Marine Biological Laboratory, Woods Hole, MA 02543, USA

RTH, 0000-0003-0004-5674

Masquerade is a defence tactic in which a prey resembles an inedible or inanimate object thus causing predators to misclassify it. Most masquerade colour patterns are static although some species adopt postures or behaviours to enhance the effect. Dynamic masquerade in which the colour pattern can be changed is rare. Here we report a two-step sensory process that enables an additional novel capability known only in cuttlefish and octopus: morphing three-dimensional physical skin texture that further enhances the optical illusions created by coloured skin patterns. Our experimental design incorporated sequential sensory processes: addition of a three-dimensional rock to the testing arena, which attracted the cuttlefish to settle next to it; then visual processing by the cuttlefish of physical textures on the rock to guide expression of the skin papillae, which can range from fully relaxed (smooth skin) to fully expressed (bumpy skin). When a uniformly white smooth rock was presented, cuttlefish moved to the rock and deployed a uniform body pattern with mostly smooth skin. When a rock with small-scale fragments of contrasting shells was presented, the cuttlefish deployed mottled body patterns with strong papillae expression. These robust and reversible responses indicate a sophisticated visual sensorimotor system for dynamic masquerade.

1. Introduction

Cuttlefish camouflage is dynamic, versatile and visually guided [1]. These marine mollusks live in visually complex environments and use a variety of camouflage mechanisms to avoid detection or recognition by predators [2]. One of those mechanisms is masquerade (figure 1*a*), a widespread tactic among animals in which a prey organism resembles an inedible or inanimate object, and is thus not recognized by predators. Many masquerading animals have evolved skin patterns and postures that are specific to their models, thus often restricting them to a specific environment [3]. Cuttlefish are not similarly constrained: not only can they adjust their colour, posture and body pattern but also, uniquely, their physical three-dimensional skin texture.

The expression of skin papillae in *Sepia officinalis* is guided by vision rather than by tactile feedback from the substrate [4], but little is known about the visual background stimuli that evoke the variable extension or retraction of skin papillae. To test this, we took advantage of the discovery that three-dimensional objects in a laboratory test arena often attract cuttlefish away from the wall and cuttlefish use the visual information on that object to select an appropriate body pattern for masquerading camouflage [5,6]. This behaviour is robust and repeatable, as shown in those experiments as well as in nature (figure 1*a*). We designed a testing arena that required cuttlefish to move a relatively long distance to a three-dimensional object when placed in the tank and retreat to its original spot when the object was removed. By sequentially placing smooth or physically textured rocks, we could then determine visual stimuli that influenced the cuttlefish's camouflage pattern and papillae expression.

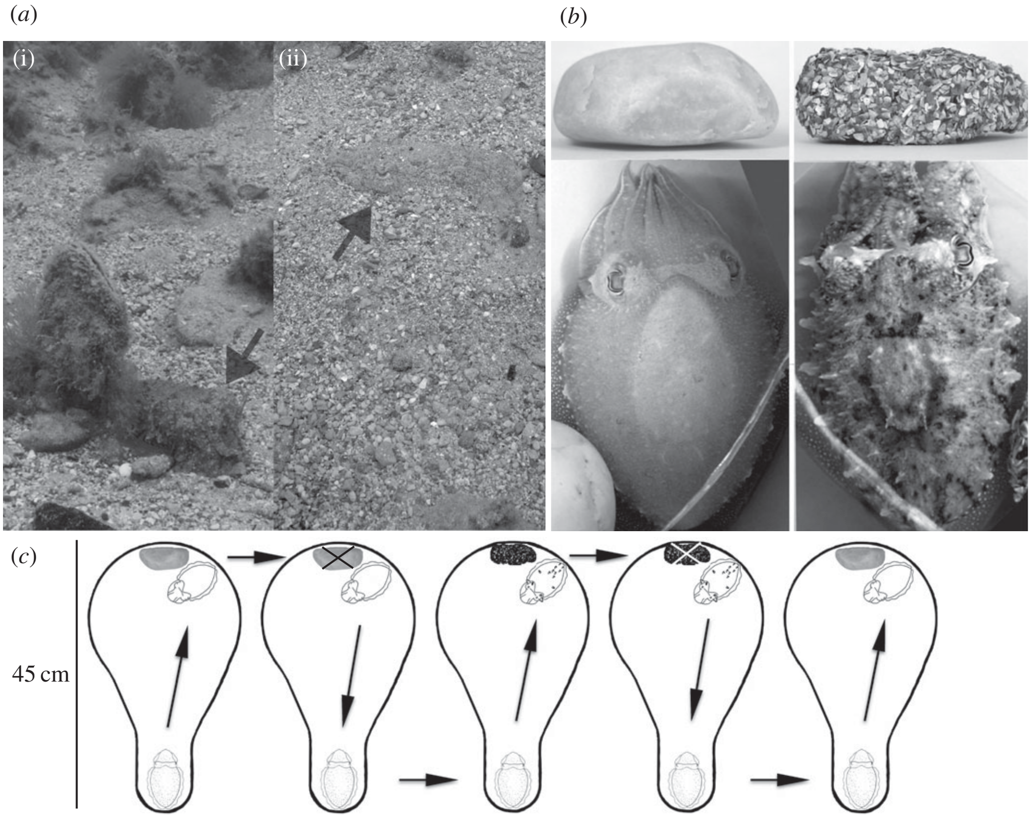

Figure 1. Dynamic masquerade in the field and laboratory. (*a*) Same cuttlefish (arrows) masquerading next to a three-dimensional shell covered in algae (i), and (ii) background matching to retard detection on sand (RTH photos, 3 m, Izmir, Turkey). (*b*) Cuttlefish in the laboratory reacting to different rocks with dissimilar expression of papillae. (*c*) Five-step behavioural sequence with presentation and removal of three-dimensional rocks of different texture. See text for details.

2. Material and methods

Field observations of *Sepia officinalis* masquerade behaviour were conducted near Vigo, Spain and Izmir, Turkey. Cuttlefish were cultured from eggs collected in the English Channel and shipped to the USA. Fifteen juvenile cuttlefish (mantle length approx. 6.5 cm) were tested in a series of three experiments. The testing arenas were supplied with continuous seawater flow and enclosed in a tent to eliminate extraneous light or movement. Animals were presented with either a smooth or textured rock, and a photo was taken when there was no body or fin movement and a consistent body pattern was shown for 2 min. The camera (Canon 5D Mark II, 100 mm lens) was attached to a monitor outside the tented chamber via HDMI cord for live view, and remotely triggered by the experimenter.

Experiment 1, conducted in a small circular tank 26 cm diameter, provided preliminary data to determine appropriate target rocks for masquerade (figure 1*b*). Experiments 2 and 3 were in a larger tank requiring a greater movement component by masquerading cuttlefish. We constructed a teardrop-shaped arena (45 cm long); all cuttlefish backed into the small cove in this arena in the absence of a rock at the other end (figure 1*c*). Once settled, a natural white smooth rock (10 × 8 × 4.5 cm) was placed at the opposite end of the arena, and the animal was allowed to resettle near the rock. Once settled, the smooth rock was removed and the animal was allowed to resettle in the small cove. Next, a similarly sized physically textured rock (made by attaching small-scale fragments of contrasting dark grey and white shells to all surfaces) was placed in the arena, the animal was allowed to resettle and the trial concluded. Experiment 3 was similarly conducted but was extended by once again placing the smooth white rock in the tank to demonstrate how robust and repeatable these behaviours were (figure 1*c*).

The following papillae types (details in [4]) were graded: major lateral eye papillae (MLEP), dorsal eye papillae (DEP), the posterior-most set of white square papillae (Pos. WSP), medium mantle papillae (MMP), the posterior-most set of paired mantle spot papillae (Pos. PMSP) and the anterior and posterior paired white triangle papillae (Ant. PWTP and Pos. PWTP), all of which are neurally controlled independently [4]. Papillae expression was graded on a scale of 0, 1, 2, 3 (figure 2*a*). 0 represented no papillae extension, 1 represented approximately 1/3 extension, 2 represented approximately 2/3 extension and 3 maximal extension. Each image was displayed on a computer monitor approximately 60 cm from the viewer and enlarged for precise scoring. Intra-observer reliability was achieved once the scores of each of the seven types of papillae in 50 random images (25 smooth rock; 25 textured rock) were consistent 90% of the time. Each image was scored at least three times randomly by the first author. Final scoring was then conducted.

3. Results

In natural microhabitats, *Sepia officinalis* masqueraded as a three-dimensional inanimate object or used the camouflage tactic of background matching to the substrate (figure 1*a*). The cuttlefish masquerading as the inanimate object in figure 1*a* has a mottle camouflage pattern that resembles not only the colour, contrast, brightness and pattern of the adjacent shell and algae, but also the rugosity and 'spikiness' of those objects by expressing its papillae to create three-dimensional physical texture. In this case, the cuttlefish can be detected by a human observer because it is a large three-dimensional object out in the open sand, but it may be difficult for a predator to recognize it as a cuttlefish. Conversely, the same cuttlefish later chose to settle in the open flat two-dimensional substrate and partially buried itself while

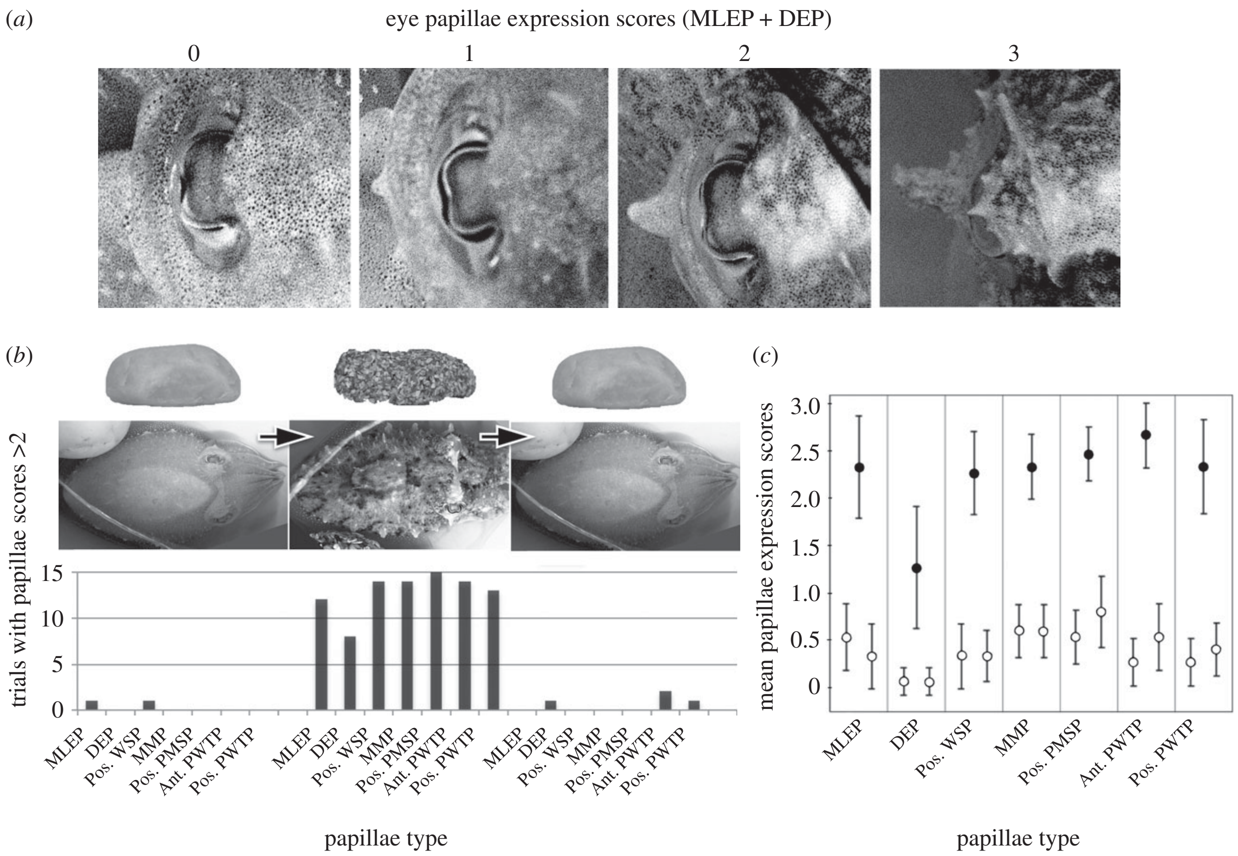

Figure 2. Sequential presentation of large rocks demonstrates dynamic masquerade. (*a*) Example scoring for expression of two of the seven papillae classes. (*b*)(i) Presentation of smooth rock attracts cuttlefish to the rock and evokes smooth skin; upon removal of the rock the cuttlefish returns to the small cove. (ii) Presentation of the textured rock attracts cuttlefish to that rock and evokes papillate skin; (iii) cuttlefish has returned to small cove and is then attracted to presentation of the smooth rock and immediately flattens its papillae. (*c*) Papillae expression scores of 15 cuttlefish for each of seven papillae types. Open circles are mean score when cuttlefish settle next to the smooth rock; filled circles are mean score when settling next to textured rock; bars are 95% CIs.

deploying a lighter mottle pattern, presumably to impair detection by predators.

In the laboratory, the 15 cuttlefish were always (i.e. 135 of 135 trials) attracted to the rock placed at the far end of the test arena. The natural white smooth rock evoked smooth skin with few papillae and a uniform pattern. Conversely, the physically textured rock consistently evoked substantial papillae expression and a mottled skin pattern (figure 1*b*).

As demonstrated in Experiment 3 (figure 2*b*), dynamic masquerade with concurrent skin patterning and papillae expression that resembled the rock was shown to be robust and repeatable. In this five-step sequential trial (figure 1*c*), each of the 15 cuttlefish showed practically no papillae expression when they swam to and settled next to the smooth rock, but all showed strong papillae expression when the textured rock was presented. To demonstrate reversibility, we then re-presented a smooth rock and the papillae scores were again close to zero. Figure 2*c* illustrates papillae expression scores for all seven papillae types for this triple succession of rocks. Mean scores of all 15 cuttlefish were significantly different for each rock (for each papillae type, a Wilcoxon signed-rank test yielded $p < 0.0002$).

Papillae change of expression was extremely fast. For a three-step change (i.e. no expression to full expression), the mean time was 1.17 s (extension range 0.46–2.83; retraction 0.23–2.03; $N = 20$ video-recorded changes). For two-step change, it was 1.03 s (0.23–2.38 extension; 0.20–2.73 retraction; $N = 36$). For one-step change, it was 0.46 s (0.23–1.19 extension; 0.27–0.90 retraction; $N = 27$).

The teardrop arena design was key to demonstrating robustness. It provided a small cove where the cuttlefish settled 100% of the time in the absence of an object. Once the smooth rock was placed opposite the cuttlefish, all swam the length of the arena, settled next to the rock and changed their skin pattern and texture to masquerade to the rock. Upon removal of the rock, all cuttlefish retreated back to the small cove (figure 1*b*,*c*). These actions were consistent with each rock placement for smooth and textured rocks.

4. Discussion

Masquerade is a common guise in many animal taxa both land and sea. Cuttlefish, like other cephalopods, have evolved rapid neural polyphenism that is visually controlled [2] and thus they have a particularly fast, complex and adaptive way to implement masquerade. Here we have drawn attention to a highly unusual feature of masquerade unknown in other phyla: morphing the physical texture of its pliable skin according to the object being imitated. Our experiments demonstrate a dual sensory process in which the cuttlefish visually detect and move to a three-dimensional shape in its vicinity and then visually assess the three-dimensional texture of that object and reproduce it in their own skin. Previous experiments have implied visual control by eliminating tactile stimulation through use of photographs of substrates or plastic barriers on top of natural substrates [4]. Our experiments provided more flexibility in decision-making by cuttlefish and indicate that cuttlefish do not need to touch the objects with their suckers (which are chemically and physically sensitive) to guide papillae expression. Some cuttlefish did touch the rocks but only briefly in Experiment 1; in the tens of trials thereafter vision was apparently guiding papillae expression. Thus there is a remote chance that some tactile feedback is used by *Sepia officinalis* although the results of Allen *et al*. [4] combined with the present results make this improbable. Octopuses also regulate papillae expression very well for various forms of crypsis (non-detection), and future research may uncover a role of tactile feedback in that taxon.

The motor output of three-dimensional skin texture is finely controlled with many degrees of freedom from completely flat (smooth) to highly rugose or papillate. Direct neural control of skin pigmentation as well as muscle control of thousands of individual papillae in the skin allow exceptionally fast change in appearance of the cuttlefish: this rapid neural polyphenism is accomplished in approximately 200–2830 ms. Polyphenism benefits masquerading peppered moth caterpillars [7] because their rarity of appearance compared with their model (in this case tree twigs) provides a frequency-dependent advantage. The speed and diversity of cephalopod polyphenism on diverse backgrounds (coral reefs, kelp habitats, rocky shores, etc.) with huge variations in rugosity also increases rarity of appearance and is likely to confer similar and perhaps additional survival advantages.

From the viewpoint of neural ecology, it proved very difficult to find natural rocks that would evoke smooth skin and highly papillate skin. For marine camouflage there are few smooth backgrounds or objects because of encrusting algae, bryozoans, tunicates, etc., and upon inspecting our extensive archive of underwater photographs we could not find an example of cuttlefish with smooth skin when masquerading as large three-dimensional objects. However, smooth skin is commonly deployed by *S. officinalis* when swimming or when stationary on the substrate (i.e. no three-dimensional objects present) and exhibiting disruptive camouflage patterns [4]. Disruptive body patterns in this species are characterized by high-contrast light and dark components of different sizes, shapes and orientation; their functions are not proven experimentally but are thought to contribute to crypsis and disruptive coloration by breaking up the recognizable outline and body parts [2]. Smooth skin may somehow enhance the optical effects of disruptive patterns but this remains unknown. Cuttlefish are highly mobile and having smooth skin while swimming provides a hydrodynamic advantage compared to many other organisms that are constrained by their elaborate fleshy projections (e.g. leafy sea dragon). Behaviourally, one of the primary functions of papillae is to obscure the outline of the animal as viewed from above or the side, thus we find papillae all over the dorsal and lateral aspects of the cuttlefish. Neurobiologically, one of the vexing questions for future investigation is how cuttlefish can assess the fine three-dimensional texture of their surrounds with monocular vision, as their eyes are opposed.

Evolutionary processes of masquerade are largely unknown [8] and dynamic cephalopod masquerade seems very specialized, yet these mollusks have invested heavily in adaptive skin that aides not only their primary defense of multiple camouflage tactics but also variable secondary defenses that require fast locomotion. This visual sensorimotor system releases them from the constraints of specific habitat selection and restricted fast motion of some other masquerading animals. But when and why are cuttlefish using masquerade versus other choices of camouflage? And what are the specific visual cues they are focusing on in the varied marine habitats to adjust their papillae with such fine control? Many questions remain, but the cephalopod system may add perspective to future studies of cognitive processes of predators and masquerading prey in the context of camouflage evolution [9].

Ethics. Cephalopods are invertebrates and not subject to USA guidelines yet the MBL staff veterinarian oversaw our animal colony and no cuttlefish were touched, stressed or injured.

Data accessibility. All photographic data available at http://hdl.handle.net/1912/8678.

Authors' contributions. R.H., K.B. and D.P.: conception and design; D.P. and K.B.: data acquisition; D.P. and R.H.: analysis and interpretation of data; D.P. and R.H.: drafting of the article. All authors revised and gave final approval of the version to be published and agree to be held accountable for its contents.

Competing interests. We declare we have no competing interests.

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