Current Biology

Report

# Multi-level control of adaptive camouflage by European cuttlefish

## Highlights

- Cuttlefish combine pattern components to camouflage themselves
- They use two distinct cognitive strategies to select these components
- One strategy matches visual features; the other categorizes the background
- These strategies can be integrated by a hierarchical motor-control system


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## In brief

Cuttlefish can express a vast range of body patterns for camouflage. Osorio et al. find that to choose the best pattern they combine matching of visual features in the background with a categorical response to their environment. This suggests how this two-level strategy can be implemented by a hierarchically organized motor system in their brain.


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# Multi-level control of adaptive camouflage by European cuttlefish

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## SUMMARY

To camouflage themselves on the seafloor, European cuttlefish *Sepia officinalis* control the expression of about 30 pattern components to produce a range of body patterns.[1] If each component were under independent control, cuttlefish could produce at least $2^{30}$ patterns. To examine how cuttlefish deploy this vast potential, we recorded cuttlefish on seven experimental backgrounds, each designed to resemble a pattern component, and then compared their responses to predictions of two models of sensory control of component expression. The body pattern model proposes that cuttlefish integrate low-level sensory cues to categorize the background and co-ordinate component expression to produce a small number of overall body patterns.[2–4] The feature matching model proposes that each component is expressed in response to one (or more) local visual features, and the overall pattern depends upon the combination of features in the background. Consistent with the feature matching model, six of the backgrounds elicited a specific set of one to four components, whereas the seventh elicited eleven components typical of a disruptive body pattern. This evidence suggests that both modes of control are important, and we suggest how they can be implemented by a recent hierarchical model of the cuttlefish motor system.[5,6]

## RESULTS

For 15 juvenile cuttlefish, we compared the pattern components (STAR Methods; Figure 1A) expressed on seven experimental backgrounds to a control uniform background. Each of these backgrounds was chosen for its visual similarity to cuttlefish pattern components. To test the two possible models of control of cuttlefish camouflage, which we call the body pattern model and feature matching model (Figures 1B and 1C), the components expressed by each animal on each background were analyzed in two different ways: first, to determine correlations between the expression of different pattern components (Figures 2 and 3), and second the dependence of component expression on specific trigger features (Figures 4 and S2).

### Correlated expression of components

A correlogram (Figure 2) of the dataset shows components whose expression was either positively or negatively correlated ($p < 0.05$ after Holm adjustment). For example, the posterior triangle, white square, and head bar (Figure 1A) are positively correlated with each other, with the posterior and anterior mantle lines, and with the median paired spots. White landmark spots are positively correlated with papillae but negatively correlated with the anterior paired spots, median mantle lines, and raised arms. Dark dots are also negatively correlated with dark median mantle lines and raised arms, while those latter two components are positively correlated with each other. Even though correlations are expected given the small range of experimental backgrounds, in no case was the correlation between components 100%, suggesting (for these pairwise comparisons) that the cuttlefish can express the components independently. Moreover, most correlations were statistically insignificant ($p > 0.05$), implying considerable independence.

To further examine correlations in the expression of individual pattern components, we used principal component analysis (PCA) of the experimental dataset.[8] The first component (PC1) explains 32% of the variation in the expression of components and PC2 16.2%. A scree plot (Figure 3A) did not readily show a change in the exponential fall-off that would indicate the number of meaningful PCs, however. Thus, for ease of depiction in a diagram, we kept the first two PCs.

PC1 is characterized by eight of the pattern components that were associated with the white square background (Figures 3B, 3C, 4, S2, and S3). Those components, coded in gray in Figure 3B, include both light (triangle, square, lateral bar, and head bar) and dark (anterior and posterior mantle lines, posterior head bar line, and median paired spots) elements. PC2 is defined by eight separate components represented in yellow and blue in Figure 3B. The three yellow components (black dots, white landmark spots, and lateral papillae) are expressed on the small white squares background. The five components coded in blue (raised arms, median mantle lines, and anterior

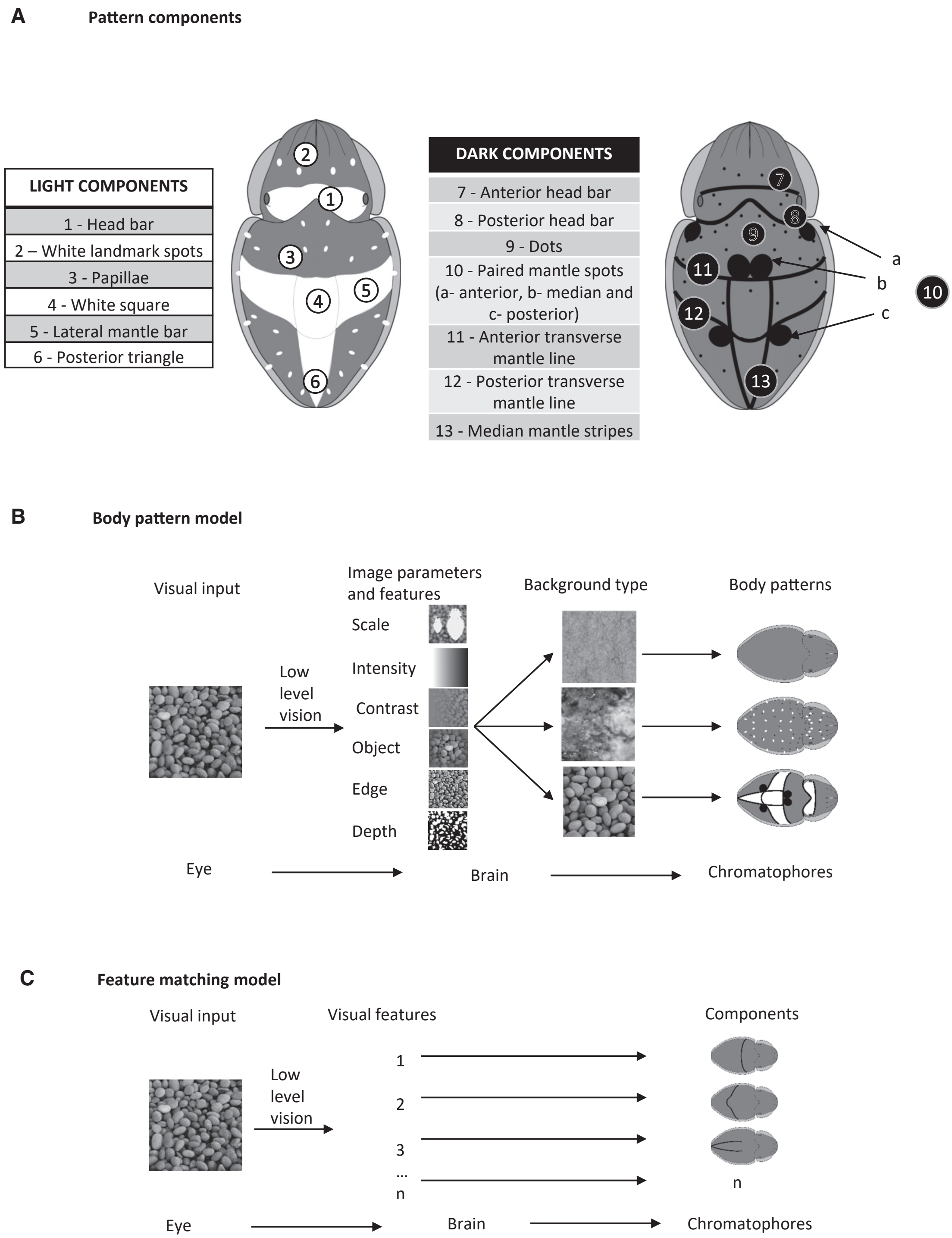


**Figure 1. Cuttlefish pattern components and models of their control**

(A) Only fifteen pattern components out of the 30 possible components[1] were elicited by the experimental backgrounds and scored in this study; in addition, the raised arms postural component was used. A component is a specific skin area, containing chromatophores, which are activated in specific way to elicit a specific localized contrast pattern.

(B) Two models of the control of cuttlefish body patterns (modified from Kelman et al.[7]). The body pattern model proposes that the cuttlefish uses a range of cues to classify the background according to its physical composition and then selects the appropriate body pattern that combines multiple components. These patterns can be adjusted according to the visual contrast and other characteristics of the background.

(C) The feature matching model proposes that each feature elicits the expression of a specific component, and the overall body pattern depends on the specific set of features present in the visual background.

See also Figure S1.

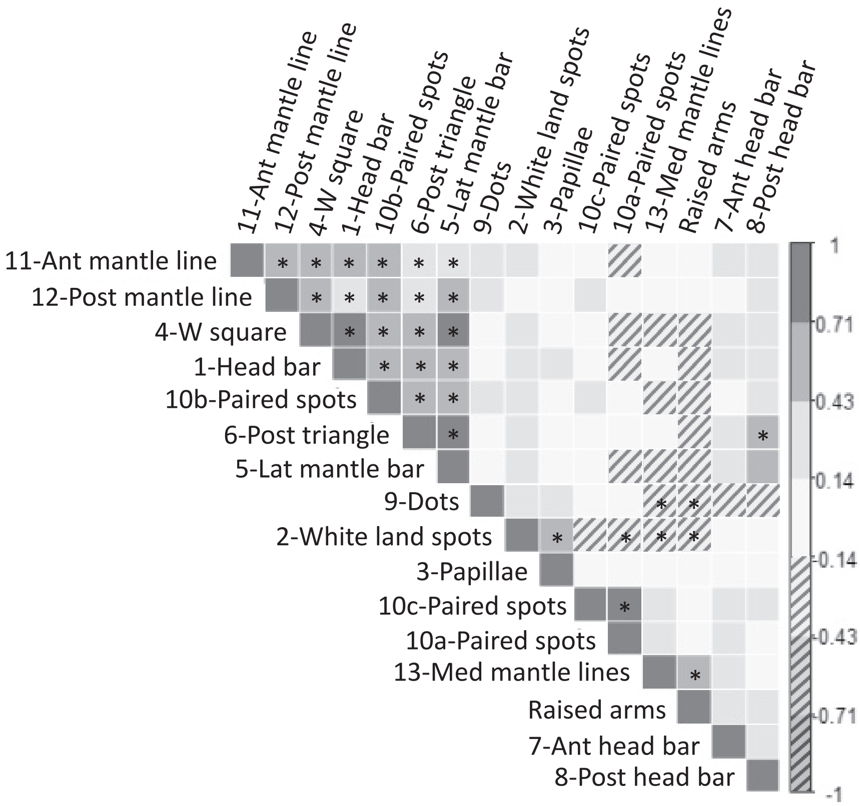


**Figure 2. Correlation coefficients for expression of components recorded**

Described in Figure 1A. Asterisks show significant correlations ($p < 0.05$) for components in our dataset.

and posterior paired mantle spots) are associated with the outlined black squares background and the black grid background. An individual factor map (Figure 3C) shows the distribution of the individual mantle patterns. The components, coded in gray, yellow, and blue, indicate the camouflage patterns associated with each of the eight backgrounds. For the body pattern model, pattern component expression should be strongly correlated (positively for the same body pattern and negatively for different body patterns) and most of the variance explained by the two first PCs, with pattern components well projected on the first factorial plane, and divided into three clear clusters. Similarly, the individual map (Figure 3C) should have three clusters, with camouflage on each background strongly associated with a cluster, on the edges of the plane. In fact, individual camouflage patterns are scattered across the plane, with no obvious clusters that might be expected in the body pattern model (Figures 1B and S1).

## Expression of individual components of experimental backgrounds

For each background, we identified components whose expression level differed significantly from that of the uniform gray background (Figure 4A), using a paired Wilcoxon signed-rank test. Each of the fifteen animals were tested once in each condition. For each experimental background, Figure 4 illustrates the component expression expected from its visual similarity to the background and the components that were displayed by the experimental cuttlefish. On small black squares (Figure 4B), there was increased expression of dark dots (Figure 1A; $V = 21$, $p < 0.05$), head bar (Figure 1A; $V = 28$, $p < 0.05$), and posterior paired mantle spots (Figure 1A; $V = 28$, $p < 0.05$). On small white squares (Figure 4C), there was increased expression of white landmark spots (Figure 1A; $V = 105$, $p < 0.001$), papillae (Figure 1A; $V = 21$, $p < 0.05$), and dark dots ($V = 62.5$, $p < 0.01$). On the black grid (Figure 4D), there was increased expression of median mantle stripes ($V = 28$, $p < 0.05$), posterior head bar ($V = 36$, $p < 0.01$), and raised arms ($V = 66$, $p < 0.01$). On white squares (Figure 4E), there was increased expression of eleven components: six light components (posterior triangle [$V = 0$, $p < 0.01$], white square [$V = 0$, $p = 0.0001842$], lateral mantle bar [$V = 0$, $p < 0.01$], white landmark spots [$V = 0$, $p < 0.01$], papillae [$V = 0$, $p < 0.05$], and head bar [$V = 0$, $p < 0.001$]) and five dark components (anterior and posterior transverse mantle lines [$V = 0$, $p < 0.05$ and $V = 0$, $p < 0.05$, respectively], median paired mantle spots [$V = 0$, $p < 0.01$], anterior head bar [$V = 0$, $p < 0.01$], and posterior head bar [$V = 0$, $p < 0.05$]). On outlined black squares (Figure 4F), there was increased expression of the anterior head bar ($V = 36$, $p < 0.05$), posterior transverse mantle line ($V = 21$, $p < 0.05$), median mantle stripes, and posterior ($V = 28$, $p < 0.05$) and anterior ($V = 45$, $p < 0.01$) paired mantle spots, but reduced expression of black dots ($V = 4$, $p < 0.05$). On white stripes (Figure 4G), there was increased expression of the white square ($V = 0$, $p < 0.01$), lateral mantle bar ($V = 0$, $p < 0.05$), white landmark spots ($V = 4.5$, $p < 0.01$), and anterior head bar ($V = 4$, $p < 0.05$). Finally, on white crosses (Figure 4H), there was increased expression of median paired mantle spots ($V = 0$, $p < 0.05$).

The components displayed by the cuttlefish often, but not always, included the pattern on which we based the background texture. Notably, backgrounds with dark features (small squares, grid, and outlined squares) elicited dark components (dots, lines, and paired spots), while backgrounds with light features (small squares, squares, crosses, and stripes) elicited both dark and light components. The expression of raised arms on the black grid (Figure 4D) compares with a previous finding that this postural component is matched to the orientation of lines in the vertical plane.[9] The number of components expressed on the background of white squares is consistent with previous findings that white squares elicit a disruptive body pattern[10,11] (Figure S2). In contrast, the background of white crosses, although visually similar to the human eye, elicited only one, which was a dark component.

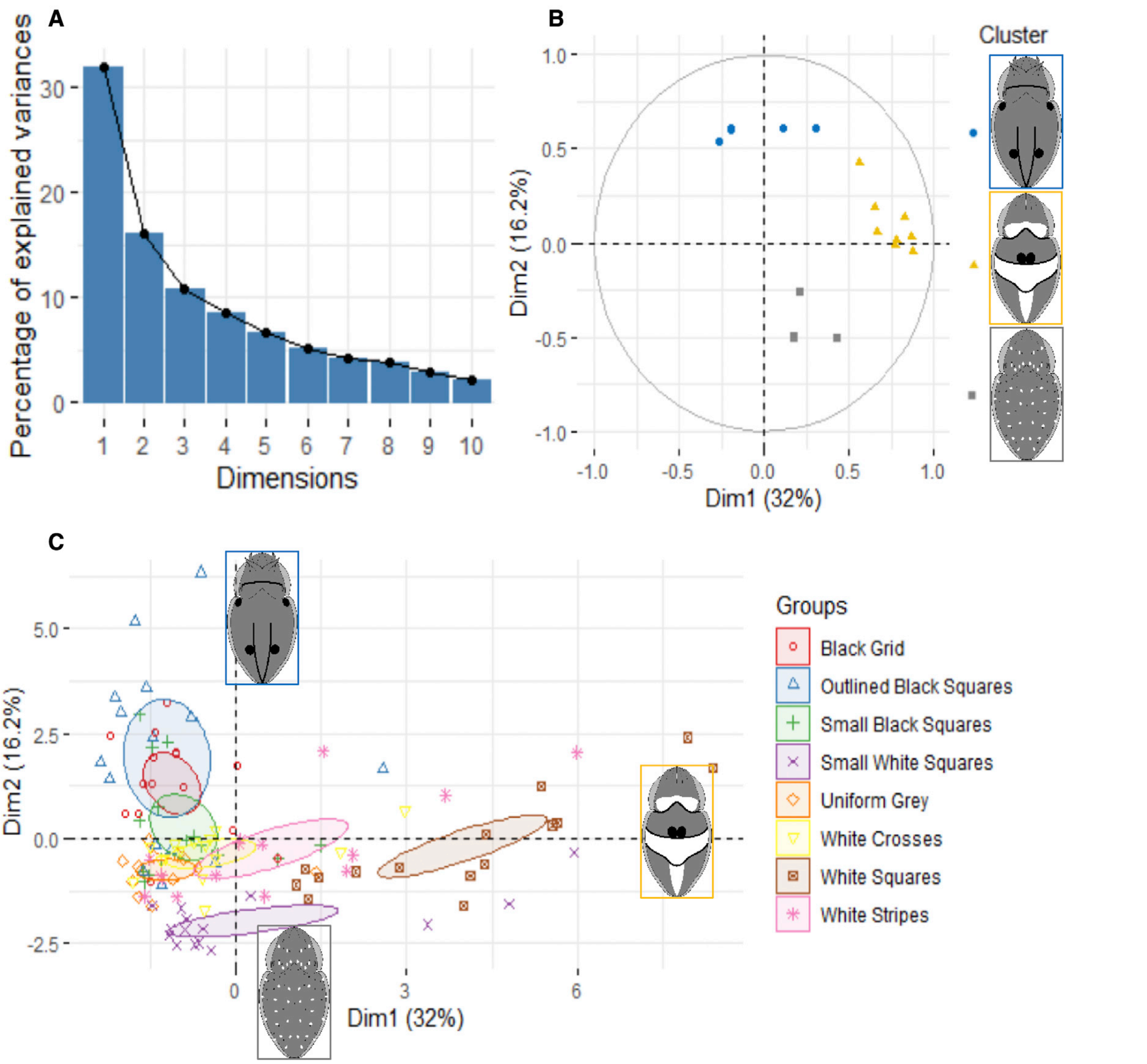


**Figure 3. Principal component analysis of cuttlefish adaptive patterning**

(A) The first two PCs account for 32% and 16.2% of the overall variability.

(B) Projections of the variables (components) on the first factorial plane. Components fall into three clusters indicated by the yellow, gray, and blue symbols, but these are not reducible to stereotyped combinations expected for the body pattern model (Figure 1B).

(C) Individual patterns are scattered across this plane, with little evidence for clumping as expected by the body pattern, and instead suggesting that components can be expressed independently of one another.

## DISCUSSION

Cryptic camouflage allows animals to match their background, but there is no established metric to define similarity for patterns or visual textures.[2,12,7] It is therefore interesting to understand how animals produce camouflage to match a particular background, especially for cephalopods such as the European cuttlefish *Sepia officinalis*, which combines about 30 pattern components to produce a range of body patterns (Figures 1 and S1). Much evidence supports a categorical account of this behavior whereby cuttlefish do not directly match the visual background, but instead select one of three body patterns according to the physical composition of the sea-floor substrate (Figure 1B; "uniform" on plain backgrounds, "mottle" on patterned surfaces, and "disruptive" in the presence of discrete objects[2,7,12,13]). Multiple visual cues elicit a shift from mottle to disruptive patterns, including the presence of edges, the size of objects, and visual depth.[3,7,14,15] A fourth pattern is expressed categorically in response to 3D objects.[12] Although widely used for interpreting cuttlefish camouflage behavior, the body pattern model (Figure 1B) does not easily explain why cuttlefish have some 30 pattern components, rather than flexibly combining a few patterns as flatfish do.[16,17] The feature matching model (Figure 1C) proposes that each component is expressed in response to one or more specific visual trigger features. The body pattern and feature matching models of adaptive coloration are not mutually incompatible, but they do make distinct predictions.

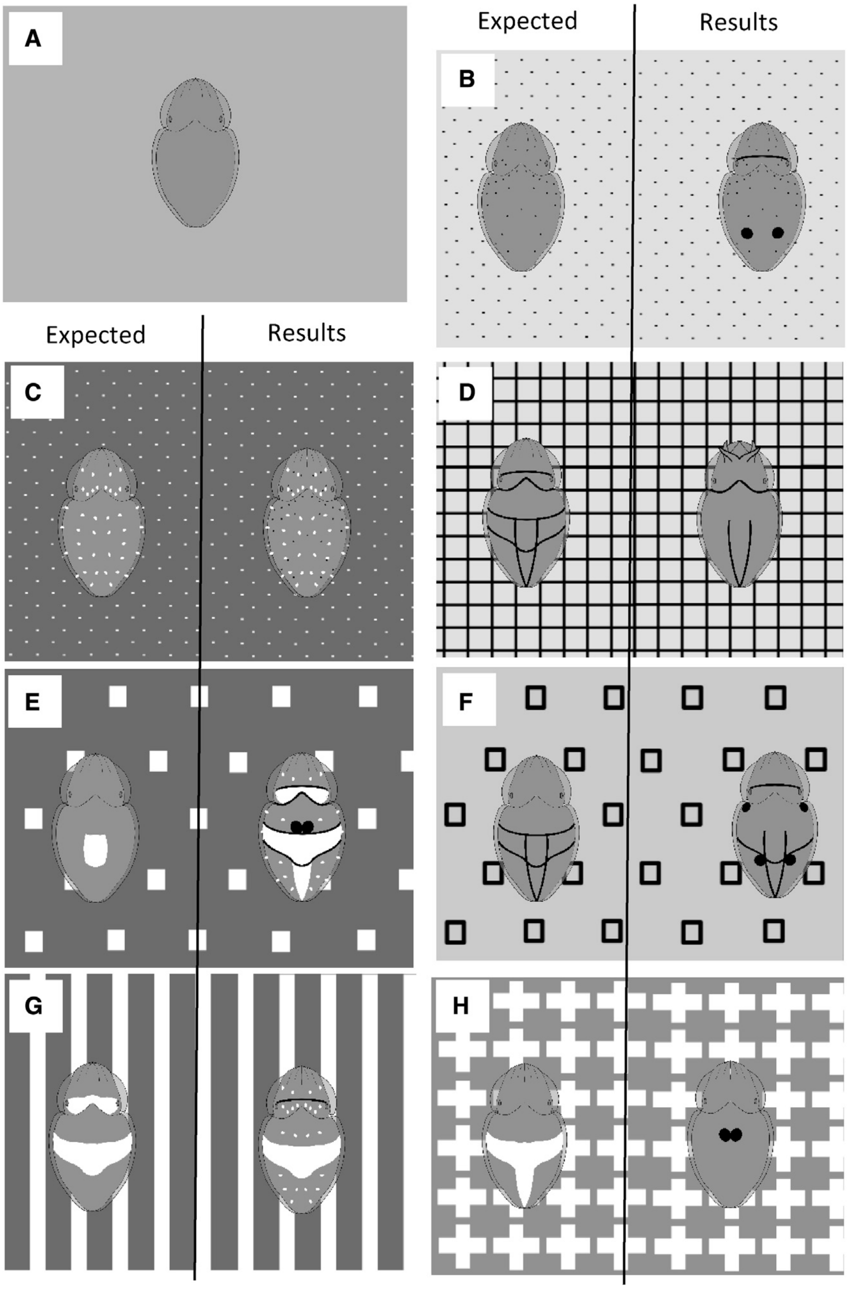


**Figure 4. The eight test stimuli**
(A) Uniform gray.
(B) Small black squares.
(C) Small white squares.
(D) Black grid.
(E) White squares.
(F) Outlined black squares.
(G) White stripes.
(H) White crosses.
Cuttlefish on the left illustrate components that visually match the substrate to the human eye. Cuttlefish on the right illustrate components whose expression is significantly elevated by the treatment in comparison to the uniform gray substrate. See also Figures S2 and S3.

Our data show that, although some pattern components are correlated, as expected for the restricted range of backgrounds used, they can be expressed independently (Figure 2). Additionally, all but one of the experimental backgrounds elicit only a small number of components (Figures 4 and S2): one background elicits one component, three backgrounds three components, and two backgrounds four components. The exception was the white square background, which elicited eleven components. This finding is consistent with previous work that finds that cuttlefish express a disruptive body pattern in response to the white squares (or circles) of about the same size as the white square component (Figure 1).[10] In fact, nine of the components identified here are shared with the eleven components associated with the disruptive pattern (Figure S2).[11]

The low percentage of variance explained by the PCA means that the dataset is not reducible to a few dimensions, as would be expected if camouflage were based on a limited number of body patterns (Figure 1B). Indeed, variables (components) and individual camouflage patterns are not well represented by the two factors identified here, as most patterns were far from the two axes and close to the center of the PCA space (Figure 3). Moreover, individual patterns are scattered across the plane, demonstrating the diversity of the 120 camouflage patterns that composed the dataset (Figure 3C). This analysis confirms that components can be expressed independently, and that they need not be combined as stereotyped body patterns.

Overall, these findings are not easily accommodated by a model in which cuttlefish categorize the background and then express one of a small number of body patterns (Figure 1B).[2,18] They are, however, broadly consistent with the feature matching model (Figure 1C), in that all but one of the backgrounds chosen elicit four or fewer components, and that a different set of components expressed is for each background (Figures S2 and S3). Camouflage would then be achieved by co-expression of multiple components, each dependent on a specific feature, with distinct body patterns being attributable to the co-occurrence of features in the background.

For a feature matching scheme to work efficiently, components should have distinct trigger features, allowing cuttlefish to match the range of natural backgrounds with a minimal number of pattern components. Such an efficient mechanism can be compared to the way in which neurons of mammalian visual cortex are thought to encode natural images.[19] Evidence in favor of the feature matching model encourages an in-depth study to identify stimuli that specifically elicit each of the pattern components. A key question is whether the features of those trigger stimuli might be better elucidated with natural stimuli than with artificial patterns. Our results (Figure 4) illustrate both similarities and differences between expected components and those that are actually expressed. This difference might arise partly because despite similarities between sensory systems, animals need not perceive stimuli as we do.[20]

Even so, it would be surprising if the cuttlefish's camouflage system was as simple as that suggested by the schematic feature matching model scheme illustrated here (Figure 1C). This study does not entirely exclude the body pattern model (Figure 1B). Notably, the white square background (Figure 4E) elicits eleven components to produce a disruptive pattern, which is consistent with a substantial literature that finds that white squares or circles elicit this pattern.[21] Moreover, previous findings that visually distinct stimuli elicit very similar responses,[4,12] and conversely, that essentially identical 2D images can produce different responses depending on the presence of physical depth cues,[7] also support the body pattern model.

### A hierarchical model of sensory motor control

A means by which cuttlefish might implement both feature matching and body pattern models of camouflage selection is suggested by recent findings on their motor system,[5,6] where analysis of correlations in spontaneous activity among 17,305 cuttlefish chromatophores implies a multi-level motor control hierarchy. The successive levels in the hierarchy corresponded to small groups of chromatophores, elements of pattern components, pattern components, and—perhaps—body patterns.

Our findings can be understood if different stimuli activate nodes at multiple levels in this control system (Figure S1). To take a putative example, the white square background (Figure 4E) might activate a high-level node that drives the expression of about eleven components, thereby producing a disruptive body pattern.[11] Conversely, white crosses background (Figure 4H), despite being physically comparable, and other backgrounds here might activate a small number of nodes, each of which drives the expression of individual lower-level components. The sub-component level nodes found by Reiter et al.[6] that control elements of the components might underlie the ability of the cuttlefish to fine-tune components, as the asymmetrical shading of the white square that apparently elicits a depth effect in response to shading cues.[22]

Hierarchical control (Figure S1) can explain how both body pattern and feature matching models (Figure 1) could be implemented in a single system. Categorization of the visual environment would be linked to the highest level of the hierarchy to generate a limited number of body patterns. In a complementary fashion, a finer feature matching mechanism would be linked to the lower levels of the hierarchy and thus would be able to generate a continuum of appearances. As Laan et al.[23] noted, cephalopod pattern control is probably evolved from locomotor systems, and such a system can be compared to the control of motor gaits in many animals, analogous to a body pattern, that can be produced by reflex and fine-tuned to display less stereotyped movements.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.04.030.

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## AUTHOR CONTRIBUTIONS

C.W.T. designed the background stimuli, all authors designed the experimental approach, F.M. and A.-S.D. conducted the experiments, all authors analyzed the data, F.M. and D.O. wrote the original draft, and A.-S.D. and D.O. wrote the revised version.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## REFERENCES

1. Hanlon, R.T., and Messenger, J.B. (1988). Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour. Phil. Trans. R. Soc. B Biol. Sci. *320*, 437–487. https://doi.org/10.1098/rstb.1988.0087.
2. Hanlon, R. (2007). Cephalopod dynamic camouflage. Curr. Biol. *17*, R400–R404. https://doi.org/10.1016/j.cub.2007.03.034.
3. Zylinski, S., Osorio, D., and Shohet, A.J. (2009). Perception of edges and visual texture in the camouflage of the common cuttlefish, *Sepia officinalis*. Phil. Trans. R. Soc. B Biol. Sci. *364*, 439–448. https://doi.org/10.1098/rstb.2008.0264.
4. Zylinski, S., Darmaillacq, A.S., and Shashar, N. (2012). Visual interpolation for contour completion by the European cuttlefish (*Sepia officinalis*) and its use in dynamic camouflage. Proc. R. Soc. B Biol. Sci. *279*, 2386–2390. https://doi.org/10.1098/rspb.2012.0026.
5. Reiter, S., and Laurent, G. (2020). Visual perception and cuttlefish camouflage. Curr. Opin. Neurobiol. *60*, 47–54. https://doi.org/10.1016/j.conb.2019.10.010.
6. Reiter, S., Hülsdunk, P., Woo, T., Lauterbach, M.A., Eberle, J.S., Akay, L.A., Longo, A., Meier-Credo, J., Kretschmer, F., Langer, J.D., Kaschube, M., and Laurent, G. (2018). Elucidating the control and development of skin patterning in cuttlefish. Nature *562*, 361–366. https://doi.org/10.1038/s41586-018-0591-3.

7. Kelman, E.J., Osorio, D., and Baddeley, R.J. (2008). A review of cuttlefish camouflage and object recognition and evidence for depth perception. J. Exp. Biol. *211*, 1757–1763. https://doi.org/10.1242/jeb.015149.
8. Kelman, E.J., Baddeley, R.J., Shohet, A.J., and Osorio, D. (2007). Perception of visual texture and the expression of disruptive camouflage by the cuttlefish, *Sepia officinalis*. Proc. R. Soc. B Biol. Sci. *274*, 1369–1375. https://doi.org/10.1098/rspb.2007.0240.
9. Barbosa, A., Allen, J.J., Mäthger, L.M., and Hanlon, R.T. (2012). Cuttlefish use visual cues to determine arm postures for camouflage. Proc. R. Soc. B Biol. Sci. *279*, 84–90. https://doi.org/10.1098/rspb.2011.0196.
10. Chiao, C.C., and Hanlon, R.T. (2001). Cuttlefish camouflage: visual perception of size, contrast and number of white squares on artificial checkerboard substrata initiates disruptive coloration. J. Exp. Biol. *204*, 2119–2125. https://doi.org/10.1242/jeb.204.12.2119.
11. Barbosa, A., Mäthger, L.M., Buresch, K.C., Kelly, J., Chubb, C., Chiao, C.C., and Hanlon, R.T. (2008). Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. Vis. Res *48*, 1242–1253. https://doi.org/10.1016/j.visres.2008.02.011.
12. El Nagar, A., Osorio, D., Zylinski, S., and Sait, S.M. (2021). Visual perception and camouflage response to 3D backgrounds and cast shadows in the European cuttlefish, *Sepia officinalis*. J. Exp. Biol. *224*, jeb238717. https://doi.org/10.1242/jeb.238717.
13. Zylinski, S., How, M.J., Osorio, D., Hanlon, R.T., and Marshall, N.J. (2011). To be seen or to hide: visual characteristics of body patterns for camouflage and communication in the Australian giant cuttlefish *Sepia apama*. Am. Nat. *177*, 681–690. https://doi.org/10.1086/659626.
14. Chubb, C., Chiao, C.C., Ulmer, K., Buresch, K., Birk, M.A., and Hanlon, R.T. (2018). Dark scene elements strongly influence cuttlefish camouflage responses in visually cluttered environments. Vis. Res *149*, 86–101. https://doi.org/10.1016/j.visres.2018.06.003.
15. Zylinski, S., Osorio, D., and Shohet, A.J. (2009). Edge detection and texture classification by cuttlefish. J. Vis. *9*, 13. https://doi.org/10.1167/9.13.13.
16. Ramachandran, V.S., Tyler, C.W., Gregory, R.L., Rogers-Ramachandran, D., Duensing, S., Pillsbury, C., and Ramachandran, C. (1996). Rapid adaptive camouflage in tropical flounders. Nature *379*, 815–818. https://doi.org/10.1038/379815a0.
17. Kelman, E.J., Tiptus, P., and Osorio, D. (2006). Juvenile plaice (*Pleuronectes platessa*) produce camouflage by flexibly combining two separate patterns. J. Exp. Biol. *209*, 3288–3292. https://doi.org/10.1242/jeb.02380.
18. Chiao, C.C., Chubb, C., and Hanlon, R.T. (2015). A review of visual perception mechanisms that regulate rapid adaptive camouflage in cuttlefish. J. Comp. Physiol. A *201*, 933–945. https://doi.org/10.1007/s00359-015-0988-5.
19. Simoncelli, E.P., and Olshausen, B.A. (2001). Natural image statistics and neural representation. Annu. Rev. Neurosci. *24*, 1193–1216. https://doi.org/10.1146/annurev.neuro.24.1.1193.
20. Caves, E.M., Nowicki, S., and Johnsen, S. (2019). Von Uexküll revisited: addressing human biases in the study of animal perception. Integr. Comp. Biol. *59*, 1451–1462. https://doi.org/10.1093/icb/icz073.
21. Chiao, C.C., and Hanlon, R.T. (2001). Cuttlefish cue visually on area—not shape or aspect ratio—of light objects in the substrate to produce disruptive body patterns for camouflage. Biol. Bull. *201*, 269–270. https://doi.org/10.2307/1543359.
22. Zylinski, S., Osorio, D., and Johnsen, S. (2016). Cuttlefish see shape from shading, fine-tuning coloration in response to pictorial depth cues and directional illumination. Proc. R. Soc. B Biol. Sci. *283*, 20160062. https://doi.org/10.1098/rspb.2016.0062.
23. Laan, A., Gutnick, T., Kuba, M.J., and Laurent, G. (2014). Behavioral analysis of cuttlefish traveling waves and its implications for neural control. Curr. Biol. *24*, 1737–1742. https://doi.org/10.1016/j.cub.2014.06.027.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Deposited data | | |
| Photographic images of cuttlefish on Experimental backgrounds | This paper | N/A |
| Raw and analyzed data files | This paper | https://doi.org/10.6084/m9.figshare.19410542 |
| Experimental models: Organisms/strains | | |
| European Cuttlefish *Sepia officinalis* | Raised from eggs, obtained locally as by-catch (laid on crab-pots) | N/A |
| Software and algorithms | | |
| R 4.0.5 | https://www.r-project.org | N/A |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Anne-Sophie Darmaillacq (anne-sophie.darmaillacq@unicaen.fr).

#### Materials availability

This study did not generate any unique reagents.

#### Data and code availability

- Raw data have been deposited at FigShare and are publicly available as of the date of publication. DOIs are listed in the key resources table.
- This paper does not report original code.

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

This study used fifteen cuttlefish (*Sepia officinalis*) raised from eggs collected along the Normandy coast.

Experiments were carried out in accordance with directive 2010/63/EU (European parliament) and the French regulations relevant to the protection and use of animals in research. All work was approved by the Normandy Ethics Committee for Animal Experimentation (CENOMEXA) and authorized by the French Ministry of Education, Research and Innovation (#22095).

### METHOD DETAILS

#### Animals

Fifteen cuttlefish (*Sepia officinalis*) were tested at the age of four months (11 ± 2 cm mantle length), at the Centre de Recherches en Environnement Côtier (CREC) of the University of Caen Normandie (Luc-sur-mer, France). The cuttlefish had been reared from eggs collected from the Normandy coast. The animals were housed individually in gray plastic tanks and moved to a tank of appropriate size as they grew (30 x 20 x 30 cm at the time of experiments) provided with a continuous flow of natural and filtered sea water. They were fed daily with locally caught sand shrimps, *Crangon crangon*, and crabs, *Carcinus maenas* and *Hemigrapsus sanguineus*.

#### Experimental setup and habituation to the apparatus

Experiments were conducted in a 60 x 40 x 30 cm tank, where cuttlefish were held in a 20 x 20 cm transparent enclosure, located in the center of the tank. The experimental tank was illuminated by a white LED strip light IP65 400 lm. Before the experiment, to habituate them to light and the transfer process, animals were placed in the experimental tank for 10 minutes a day for three consecutive days.

#### Experimental substrates

Experimental substrates included a uniform gray control (nominally 65% gray) designed to elicit a uniform body pattern (Figure 4A) and seven patterns that were designed to mimic the respective features of several pattern components (Figure 1A): i) Small black squares on a gray background (85% gray) to elicit a pattern of dark dots (Figure 4B); ii) Small white squares on a gray background (35% gray) to elicit a pattern of light spots (Figure 4C); iii) A black grid on a gray background (85% gray) to elicit median and transverse black lines (Figure 4D); iv) White squares on a dark background (35% gray) to elicit the mantle white square (Figure 4E), and a density similar to that used by Chiao and Hanlon;[10,21] v) Outlined black squares on a gray background (75% gray) to elicit black lines around the central mantle rectangle (Figure 4F); vi) White stripes on a dark background (35% gray) to elicit two transverse stripes formed by the white square, white mantle bar, and white head bar, or a longitudinal stripe including the central white square (Figure 4G). vii) A gray background (35% gray) with white crosses to elicit white square, white mantle bar, and white triangle. The size of the features in each pattern were matched to those in animals of average size.

The background substrates were laminated, to be waterproof, and placed on the test arena floor inside the tank.

## QUANTIFICATION AND STATISTICAL ANALYSIS

The running order for testing the fifteen cuttlefish on the seven substrates was pseudorandomized. Ten images of the body pattern were taken over a period of ten minutes with an Olympus Tough TG-6 camera. If an animal did not settle within ten minutes, the trial was repeated another day. The last settled body pattern was selected for analysis. Images of the animals were excised from the background to avoid bias during grading. Each of the 120 images was graded manually by a single observer for the expression of 16 pattern components (Figure 1A) on a four-point scale, from 0: not expressed to 3: strongly expressed,[8] resulting in a dataset of 1920 ratings.

One observer (FM) blindly scored all the images. Interobserver reliability was checked using a sample of 10 images scored by two independent observers (FM and BL). The percentage of agreement was 78.1% according to Cohen's kappa, which is good.

Components were those described by Hanlon & Messenger[1] with some modifications. For example, the component described by Hanlon & Messenger as 'paired mantle spots' was expanded to separately score anterior, median and posterior paired mantle spots (Figure 1A), as the intensity of expression of these dark spots can vary independently.[7] One of the 16 displayed components was postural: raised arms. Other components were absent or too rare to be analyzed.

All the statistical analysis was made using R software (R version 4.0.5). Given the small sample size, correlations between components were assessed by the Spearman rank correlation coefficient (Figure 2). Probability values were adjusted for multiple comparisons by the Holm method. To aid understanding of the camouflage behavior, the resulting data set was reduced using a principal components analysis (PCA; Figure 3). Differences in the expression of pattern components, in comparison to those displayed on a uniform gray background (Figure 4; see also Figure S2), were assessed by a paired Wilcoxon signed-rank test.