Effects of early visual experience on the background preference in juvenile cuttlefish Sepia pharaonis

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Although cuttlefish are capable of showing diverse camouflage body patterns against a variety of background substrates, whether they show background preference when given a choice of substrates is not well known. In this study, we characterized the background choice of post-embryonic cuttlefish (Sepia pharaonis) and examined the effects of rearing visual environments on their background preferences. Different rearing backgrounds (enriched, uniformly grey and checkerboard) were used to raise cuttlefish from eggs or hatchlings, and four sets of two-background-choice experiments (differences in contrast, shape, size and side) were conducted at day 1 and weeks 4, 8 and 12 post-hatch. Cuttlefish reared in the enriched environment preferred high-contrast backgrounds at all post-embryonic stages. In comparison, those reared in the impoverished environments (uniformly grey and checkerboard) had either reversed or delayed high-contrast background preference. In addition, cuttlefish raised on the uniformly grey background, exposed to a checkerboard briefly (0.5 or 3 h) at week 4 and tested at week 8 showed increased high-contrast background preference. Interestingly, cuttlefish in the enriched group preferred an object size similar to their body size at day 1 and week 4, but changed this preference to smaller objects at week 12. These results suggest that high-contrast backgrounds may be more adaptive for juvenile cuttlefish, and visually enriched environments are important for the development of these background preference behaviours.

Keywords: enriched environment; impoverished environment; background contrast; behavioural plasticity

1. INTRODUCTION
Camouflage is one form of primary defence in nature. Animals with fixed body patterns tend to choose appropriate backgrounds for concealment. For example, it is known that selection of the background is critical for cryptic moths [1]. At the population level, habitat selection is an important factor in animal dispersal, and induction of a natal habitat preference has been observed in a variety of animals with potential adaptive significance [2]. Although genetic variation for habitat preference has been well documented [3], a previous study using the Xenopus laevis tadpole showed that visual experience can affect background preference [4]. A recent study further demonstrated that changing visual input by altering light exposure regulates X. laevis camouflage coloration in response to illumination [5].

Adaptive body coloration in cephalopods is among the most sophisticated in the animal kingdom because the neurally controlled chromatophore system [6] permits a diverse repertoire of body patterning for camouflage [7]. Cuttlefish are capable of showing distinct camouflage body patterns against a variety of background substrates, but whether they have a preference for a certain background type in their habitats is not well known. An earlier study examining sub-adult cuttlefish’s camouflage pattern preference in Sepia officinalis suggests that there is no strong substrate preference when given the choice of various natural or artificial backgrounds [8]. However, whether early visual experience in their rearing environments would affect background choice in post-embryonic cuttlefish remains to be studied.

Early visual experiences can influence several cuttlefish behaviours, including learning/memory, prey preference and body pattern maturation [9–12]. In particular, it has been shown that environmental enrichment can enhance the development of memory and body pattern in S. officinalis [10,12]. In a previous study, we found that S. pharaonis cuttlefish raised in a high-contrast background took significantly longer time to settle down on a uniform substrate than on a checkerboard [11]. This observation that rearing environments affect cuttlefish’s acclimation time on different substrates suggests the possibility that early visual experience may alter juvenile cuttlefish’s background preference. To test whether cuttlefish indeed have preferences for certain backgrounds, which themselves are affected by early rearing conditions, visually enriched and impoverished environments were used to raise cuttlefish from eggs or hatchlings, and their background preferences were tested at day 1 and weeks 4, 8 and 12 post-hatch. Complementary to these long-term exposures (days or weeks), the effect of brief exposure to a high-contrast substrate (minutes or hours) was also examined for animals reared in a low-contrast substrate, in order to study their behavioural plasticity.

2. MATERIAL AND METHODS
Eggs of S. pharaonis were separated into four cohorts at least seven days before hatching. The first cohort was raised in tanks consisting of light/dark rocks and artificial algae on a sandy bottom (enriched group, or E group), the second one was reared on a uniform-grey background (low-contrast group, or L group), the third one was kept on a black/white checkerboard background (high-contrast group, or H group) and the fourth one was placed in blue perforated plastic containers (standard group, or S group). These four cohorts of animals were tested on day 1 post-hatch (experiment 1). In a different set of experiments, cuttlefish from the S group were divided into three cohorts immediately after hatching, and reared in either an enriched (E group) or an impoverished (L and H groups) environment until being tested at week 4, 8 or 12 (experiments 2 and 3). All developing embryos and hatchlings were group-reared in this study (see electronic supplementary material, table S1 for details of rearing conditions).
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Figure 1. Experimental device and test backgrounds. (a) The experimental device consisted of a rectangular arena with an entry chamber in a large seawater tank. Arrowhead indicates the water level. (b) Four pairs of backgrounds for testing cuttlefish's preference: (b(i)) contrast preference; (b(ii)) shape preference; (b(iii)) size preference; (b(iv)) side preference.

Four sets of two-background-choice experiments were performed to examine cuttlefish’s preference for visual features (figure 1). Contrast and size of background pattern are known to be important visual features for cuttlefish camouflage behaviour [7]. Cuttlefish can distinguish different shapes, so the shape preference test served as a control. The side preference test was used as another control to examine whether there is any turning preference. In each trial, two backgrounds were presented side-by-side for an animal to choose. Individual cuttlefish were placed in an opaque chamber before experimentation. After the door was raised, cuttlefish were allowed to enter the arena head first and freely move around, and the entire testing arena was video-recorded for 50 min. To sample data objectively, one 2 s video clip was acquired for every 30 s, thus yielding 100 video clips per animal per trial.

To gauge stability of background preference behaviour, each video clip was manually graded according to the following criteria: score 1, if cuttlefish were swimming; score 2, if cuttlefish stayed at the bottom of the arena with fin movement; score 3, if cuttlefish settled down and showed no sign of fin movement. The scores of animal response on both sides of the arena and in-between were obtained from grades of 100 clips. The total scores were tallied and only those animals with a total score above 150 (half of the maximum 300) were included in the results, in order to ensure stable responses.

In experiment 1, hatchlings from E, L, H and S groups were tested for their background preferences on day 1. Each cuttlefish was tested only once to ensure that hatchlings had never seen other visual stimuli before the background preference test. In experiment 2, animals from E, L and H groups were tested at weeks 4, 8 and 12. Each cuttlefish was repeatedly tested for four sets of background preferences in the sequence contrast, shape, size and side on separate days, in order to minimize the contrast priming effect and to reduce the number of animals used in this experiment. Animals tested at three developmental stages were different cuttlefish. In experiment 3, cuttlefish from the L group were intentionally exposed to a checkerboard background for only 0.5 or 3 h in group at week 4, and repeatedly tested for four sets of background preference tests at week 8, in the same order as in experiment 2 and on separate days. These animals were different individuals from those used in experiment 2. Note that two backgrounds in each trial were presented side-by-side in a randomized manner in experiment 1 to avoid the innate side preference. However, the fact that cuttlefish were group-reared (i.e. individuals could not be identified) and repeatedly tested for four background preferences in experiments 2 and 3 prevents us from randomizing two backgrounds in each trial, thus the side of a small checkerboard background (used in all four tests) was fixed in each trial at right, left and right in the contrast, shape, size and side preferences, respectively. The numbers of cuttlefish used in each test are shown in the electronic supplementary material, table S2.

The one-tailed Friedman test followed by the Holland–Wolfe post hoc comparison [13] were used to evaluate if cuttlefish show background preference based on the rank of response scores on two sides and the middle of the arena (experiments 1 and 2). To determine whether L-group animals exposed to a checkerboard briefly at week 4 alter the trend of their background preferences at week 8, the Jonckheere–Terpstra test was used (experiment 3) [14]. All statistical tests were performed using R software.

3. RESULTS

(a) Experiment 1

When hatchlings were tested on day 1, only those animals whose embryos were reared in a visually enriched environment (E group) showed a significant high-contrast background preference (figure 2a(i)). Cuttlefish raised prenatally in visually impoverished environments (L, H and S groups) did not have background contrast preferences immediately after hatching. Interestingly, only the E group hatchlings had a significant object size preference on day 1 (figure 2a(iii)). Most cuttlefish (E, L and H groups) did not have shape and side preferences within 24 h of hatching, with the exception of the S-group hatchlings in the shape test (figure 2a(ii,iv)).

(b) Experiment 2

Cuttlefish raised in a visually enriched environment showed significant high-contrast background preferences at weeks 4, 8 and 12 post-hatch (figure 2b(i), 2c(i), 2d(i)). However, the L-group cuttlefish preferred a low-contrast background at week 8, and the H-group animals did not show a high-contrast background preference until week 12.

Interestingly, the E-group cuttlefish preferred a checkerboard with large check size at week 4 (figure 2b(iii)) but showed an opposite preference at week 12 (figure 2d(iii)). In comparison, animals reared in an impoverished environment (L and H groups) did not have this size preference (figure 2b(ii), 2c(ii), 2d(ii)). There were no shape and side preferences in E-, L- and H-group cuttlefish at any post-embryonic stages (figure 2b(ii,iv), c(ii,iv), d(ii,iv)).

(c) Experiment 3

When the L-group cuttlefish were exposed to a checkerboard for 0.5 or 3 h at week 4 and tested at week 8, it is apparent that pre-exposure significantly increased their high-contrast background preference (figure 2e(i)). There were no shape, size and side preference changes in this pre-exposed group of animals (figure 2e(ii)–(iv)).

4. DISCUSSION

Cephalopods can dynamically change their skin colorations for camouflage [7]. Unlike animals with fixed

body patterns, a substrate preference in cephalopods does not seem to be necessary. The previous study on substrate choice in *S. officinalis* showed that cuttlefish do not have substrate preference for camouflage [8]. However, we found that juvenile cuttlefish *S. pharaonis* reared in a visually enriched environment preferred a high-contrast background. In nature, higher-contrast substrates are often complex environments, and it is known that animals camouflage more effectively in heterogeneous than homogeneous visual backgrounds [15]. In addition, *S. pharaonis* inhabit diverse environments [16], so that this experience-dependent high-contrast background preference in juvenile cuttlefish may provide a defensive advantage early in their life.

Cross examination of cuttlefish body size and check size at different developmental stages revealed that the E-group animals seemed to prefer the background with object size similar to their body size at day 1 and week 4, but changed this preference to smaller objects at week 12 (electronic supplementary material, table S1). This observation suggests that juveniles may switch their camouflage strategy from masquerade to background matching during development [17]. The fact that most cuttlefish did not have a shape preference also corroborates the previous finding that cuttlefish cue visually on area, not shape, of light objects in the substrate, in order to produce camouflage body patterns [18].

It is well known that environmental enrichment is important for neural development [19]. In cuttlefish, embryonic sensory experience was found to affect prey preference [20,21]. Our finding that embryonic exposure to a visually enriched environment is required for cuttlefish’s background contrast preference at postnatal day 1 suggests that early visual experience in the prenatal period is critical for shaping the background preference behaviour later on. In addition, the present result that impoverished environments (uniformly grey and checkerboard) reversed or delayed the cuttlefish’s background contrast preference seen in cuttlefish raised in an enriched environment also supports the notion that visual complexity is crucial for proper development of cuttlefish behaviour [10]. Note that, although the checkerboard provides a high-contrast and repetitive pattern, it is flat and homogeneous in

![Figure 2](http://rsbl.royalsocietypublishing.org/)}
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object size, thus it is distinctly different from enriched environments and should be considered as an impoverished substrate. This result is also consistent with a previous finding that visually enriched environments facilitate body pattern maturation in cuttlefish [12], and suggests that cuttlefish may rely on previous experience to choose an appropriate background for camouflage. Furthermore, the observation that brief exposure to a high-contrast substrate at week 4 can influence cuttlefish’s background preference at week 8 confirms the evidence for behavioural plasticity in developing cuttlefish, and also highlights the importance of learning and memory in modifying subsequent behaviours [9–12].

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