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Effects of Visual Cues of a Moving Model Predator on Body Patterns in Cuttlefish *Sepia pharaonis*

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We examined the effects of predator–prey distance (PPD) and trajectory of the predator on the body patterns that the pharaoh cuttlefish, *Sepia pharaonis*, shows in response to a predator. A model predator moving in three different trajectories was presented to the cuttlefish: T1, approached the cuttlefish but bypassed above; T2, approached directly toward the cuttlefish; T3, bypassed the cuttlefish both vertically and horizontally. We divided the body patterns that the cuttlefish expressed into seven categories, i.e., "uniform light", "disruptive", "center circle", "dark square", "vertical stripe", "all dark" and "eyespots". In T1, the number of individuals that showed "dark square" increased as the model approached the cuttlefish, whereas the number of individuals that showed "disruptive" decreased. In T2, the number of individuals that showed "all dark" and "eyespots" increased as the model approached the cuttlefish. In T3, the number of individuals that showed "dark square" and "vertical stripe" increased as the model approached the cuttlefish, and it tended to decrease as the model receded from the cuttlefish. These results demonstrate that *S. pharaonis* changes its body patterns according to PPD and the trajectory of the predator, which would affect predation risk and/or predator perception.

Key words: cuttlefish, body pattern, primary defense, predator-prey distance, predator trajectory

INTRODUCTION

Coleoid cephalopods (squid, cuttlefish and octopus) have the most sophisticated ability to change their appearance in the animal kingdom (Hanlon et al., 1999; Mäthger et al., 2008; Chiao et al., 2009). Their body patterns are formed by a neurally-controlled pigmented chromatophore system, structurally-reflecting iridophores, and light-scattering leucophores in the skin, which enable them to change skin colors and patterns drastically and immediately (Messenger, 2001). Many field observations and laboratory experiments have shown that the body patterns of cephalopods change according to given situations, especially in anti-predator contexts (e.g., Moynihan and Rodaniche, 1982; Hanlon and Messenger, 1988, 1996; Mather, 2010; Staudinger et al., 2011). Because cephalopods lack hard structures such as shells and spines for defense against predators, they use their body pattern-changing ability to avoid predation by various species (e.g., marine mammals, diving birds, teleost and elasmobranch fish) (Hanlon and Messenger, 1996; Hanlon et al., 2009).

Although it is widely known that cuttlefish show various body patterns in response to predators, the specific functions of many body patterns are still not fully understood (see Hanlon and Messenger, 1996; Staudinger et al., 2011). To evaluate the function of each body pattern, it is necessary to elucidate the relationship between a given body pattern and stimuli that may evoke it. Recently, the stimuli that induce cuttlefish to display eyespots on their posterior mantle have been examined (Langridge et al., 2007; Langridge, 2009). Langridge et al. (2007) showed that *Sepia officinalis* displays "eyespots" only towards juvenile sea bass (*Dicentrarchus labrax*), a visually hunting predator, and never towards crabs (*Necora puber*) or juvenile dogfish (*Scyliorhinus canicula*), chemically hunting predators. However, even in response to crabs and juvenile dogfish, cuttlefish continuously change their body pattern and express diverse patterns other than "eyespots" (see Langridge, 2009; Staudinger et al., 2013). Thus, factors other than predator type may also affect body patterns that cuttlefish show in anti-predator contexts.

Predation risk has been suggested to affect when prey animals show alternative anti-predator behaviors that serve different functions (Lima and Dill, 1990; Caro, 2005). For example, prey animals do not escape immediately after detecting an approaching predator, but only after the cost of staying exceeds the cost of fleeing (see Ydenberg and Dill, 1986; Stankowich and Blumstein, 2005), that is, after the predation risk becomes very high. Cooper (2010) showed that a lizard, *Callisaurus draconoides*, displays a pursuit deterrent signal to a predator most frequently when predation risk is intermediate, as prey needs not signal when predation risk is very low and should immediately escape when

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this risk is very high. Based on the findings of Ydenberg and Dill (1986) and Stankowich and Blumstein (2005), we predicted that cuttlefish would change body patterns depending on the level of predation risk, even in response to the same type of predator (see also Mather, 2010).

Predator-prey distance (PPD) and the trajectory of an approaching predator are good indicators of predation risk for prey: the closer the predator approaches the prey, the more likely the prey is to be detected and captured by the predator (Broom and Ruxton, 2005). The trajectory of a predator also affects predation risk, as the more directly the predator approaches the prey, the more likely it is that the predator has detected or will detect the prey (Cooper, 2009).

Previous researches using a real predator have shown that cuttlefish and squid change their body patterns according to PPD (Langridge et al., 2007; Mather, 2010). However, those studies did not examine how cephalopods change their body patterns with continuously changing PPD. In addition, it is difficult to evaluate the confounding effect of the movement of a predator when using a real animal.

In the present study, we examined the effects of PPD and the predator's trajectory on the body patterns of a cuttlefish, *Sepia pharaonis* Ehrenberg, 1831, using a model predator as a stimulus. This method enabled us to experimentally control the movement of a predator and examine how visual cues affect anti-predator behavior of *S. pharaonis*. We first describe and define body patterns exhibited by *S. pharaonis* during the experiment. We next examine the effects of PPD and the trajectory of the approaching predator on its body patterns.

MATERIALS AND METHODS

Subjects

Forty-eight *S. pharaonis* (78–98 mm in mantle length) were used in the present study. Eggs of *S. pharaonis* were collected from inshore waters of Okinawa Island, Japan in May 2011 and were transported to the Senbaru campus of the University of the Ryukyus, where they were reared until September. Before the experiments, subadult cuttlefish were transported to Sesoko Station, Tropical Biosphere Research Center (TBRC) of the University of the Ryukyus. Cuttlefish were maintained in six round tubs (70 cm in diameter, 30 cm in water depth), with eight individuals per tub. We were able to identify cuttlefish in each tub by small black spots on their mantle; the intensity of expression of these spots changed but their locations on the mantle were stable (see also Byrne et al., 2010).

Predator stimulus

Several previous studies demonstrated that *S. officinalis* uses different anti-predator behaviors according to the species of predators (e.g., Hanlon and Messenger, 1996; Langridge et al., 2007), which may suggest that the anti-predator behaviors of cuttlefish are affected by the appearance of predators. Therefore, we made a model that resembles a real predator as a predator stimulus in this study. A 50-cm-long model predator was made from foamed styrene (Fig. 1). This was modelled after a spangled emperor, *Lethrinus nebulosus*, a fish that is abundant around Okinawa Island (Carpenter and Allen, 1989) and is considered to be an active predator that preys on various animals such as mollusks (including cephalopods), crustaceans and sea urchins (Kiso and Kosuge, 2007). We added a 4 kg weight to the model so that it could remain submerged.

Fig. 1. The model predator (Total length = 50 cm) used in the experiments, modelled after a spangled emperor, *Lethrinus nebulosus.*

Experimental setup

All experiments were conducted in a circular tank (300 cm in diameter, 110 cm in water depth) in November and December 2011 at Sesoko Station, TBRC. A polyvinyl chloride (PVC) pipe above the middle of the experimental tank was used as a structure from which the model predator is suspended. The model predator was connected to four rings on the PVC pipe with fishing line and hung from the pipe. We moved the model along the pipe by pulling a string attached to the rings; at no time could the cuttlefish see the experimenter. The model was initially placed behind a white plastic board (50 × 50 cm), and thus a cuttlefish was not able to see it before the experiment started. A cuttlefish was introduced into a transparent cylindrical arena (30 cm in diameter, 60 cm in height) to maintain its approximate position during the experiment. The substrate was the bare bottom of the tank, which was basically uniform white. The arena was placed adjacent to the inner side of the wall of the experimental tank. The response of cuttlefish to the moving model predator was recorded by a video camera (GoPro HD HERO) mounted above the arena but underwater to avoid disturbance of the video recording by the flickering water surface. The cuttlefish was given 15 min to acclimatize after its introduction into the arena. We initiated the experiment only when the cuttlefish settled on the bottom of the tank and its body pattern became stable. Each experiment was initiated by removing the plastic board. Thirty seconds after the removal of the board, an experimenter started pulling the model predator with approximately consistent velocity (approximately 10 cm/s). The average time taken for each trial was 22.5 s. Cuttlefish changed their body patterns immediately after the removal of the board, but the patterns became fairly stable within 30 s. We moved the model in three different trajectories. Trajectory 1 (T1): the model predator was hung 70 cm from the bottom of the experimental tank, and the arena was placed so the model predator approached straight toward the cuttlefish (Fig. 2A). Trajectory 1 was intended to imitate a trajectory such that when the predator approaches the cuttlefish but bypasses it above. Trajectory 2 (T2): the model predator was hung 30 cm from the bottom of the experimental tank, and the arena was placed so that the predator approached directly toward the arena (Fig. 2A). Trajectory 2 was intended to imitate a predator directly approaching the cuttlefish. Trajectory 3 (T3): the height of the model predator was the same as that of T1, but the pipe was rotated 90° (Fig. 2B). Trajectory 3 was intended to imitate a trajectory in which the predator bypasses the cuttlefish both vertically and horizontally. Because cuttlefish have high visual acuity, a wide field of view (see Watanuki et al., 2000; Mäthger et al., 2013) and highly mobile eyes (Budelmann and Young, 1993), we consider that the cuttlefish were able to perceive the location and the movement of the model predator accurately. Each cuttlefish was used once for

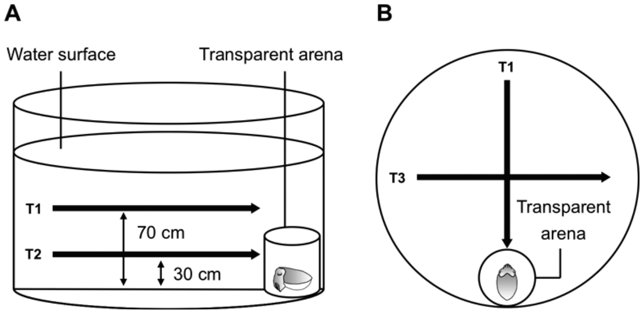

Fig. 2. Three trajectories **(A)** T1, T2 and **(B)** T3 of the model predator presented to *Sepia pharaonis* in the experimental arena. **(A)** Lateral view, **(B)** Top view.

each trajectory and was subjected to all three trajectories in random order with a minimum of a four-day interval between two trials.

Data analysis

To examine body patterns that *S. pharaonis* expressed in the absence of the predator stimulus in the experimental tank, we recorded its body patterns for 30 s prior to removing the board (control period). From the recorded videos, we extracted images of cuttlefish body patterns every 6 s, and a total of five images was extracted from each trial.

To examine how *S. pharaonis* changed body patterns according to the movement of the model within a trajectory, we extracted video images of its body patterns in the following five stages for T1 and for T2: 1) 3 s before the model predator began to move, and when the linear PPD, that is, the straight-line distance between the cuttlefish and the model predator, was 2) 190 cm, 3) 150 cm, 4) 110 cm and 5) 70 cm. These positions were estimated from the diameter of the tank (300 cm), the distance the model moved and the height from the bottom (Fig. 3). For T3, we extracted video images of body patterns of the cuttlefish in six different stages: 1) 3 s before the model predator began to move, and when the model predator moved 2) 40 cm, 3) 80 cm, 4) 120 cm, 5) 160 cm and 6) 200 cm. The linear PPD was shortest at 120 cm. The linear PPD at 80 cm and 160 cm would be similar. Likewise, the linear PPD at 40 cm and 200 cm would be similar.

Using the extracted video images, we classified the body patterns of *S. pharaonis*. Hanlon and Messenger (1988) suggested that body patterns of cuttlefish should be classified hierarchically based on the chromatic components constructing each body pattern. They identified 34 chromatic components in *S. officinalis* and classified its body patterns into 13 categories (Hanlon and Messenger, 1988). Because the chromatic components have not been identified in *S. pharaonis*, we used the repertoire of chromatic components of *S. officinalis*, which shows body patterns similar to *S. pharaonis* (Shohet et al., 2007) and shares many chromatic components with it.

In each stage, we calculated the proportion of individuals that showed each body pattern by dividing the number of individuals expressing the body pattern by the total number of individuals used in the experiment (n = 39, see Results). To statistically analyze whether *S. pharaonis* changed body pattern while the model was moving within each trajectory, we compared the proportion of the individuals showing each body pattern at each stage within the trajectory using Cochran's Q test. Additionally, to examine whether *S. pharaonis* changed body patterns according to the trajectory of the approaching predator, we compared the proportion of individuals that showed each body pattern in response to T1 with that in response to T2 when the linear PPD was the same using McNemar's test with Bonferroni-corrected P values (0.05/5) for multiple comparison.

To examine how each cuttlefish changed body patterns in response to the movement of the model predator, we counted the number of transitions of body patterns of each cuttlefish. For T1 and T2, transition from stage 1 to stage 5 was counted. For T3, transition from stage 1 to stage 3 (i.e., when the model predator was approaching the cuttlefish) and that from stage 4 to stage 6 (i.e., when the model predator was retreating from the cuttlefish) were counted. We did not conduct statistical analysis on these data to examine whether the transitions of body patterns were significantly non-random, because the sample size was too small.

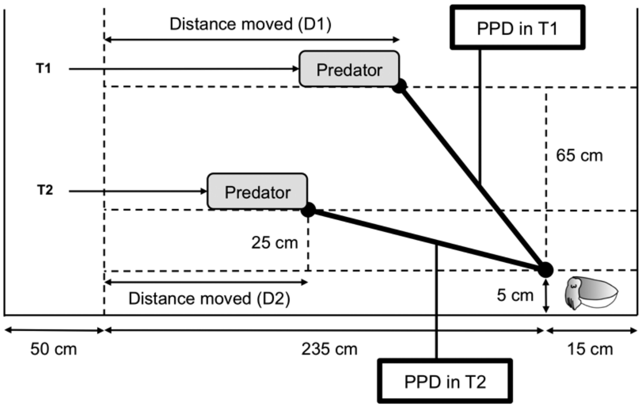

Fig. 3. Variables used for estimating the positions (i.e., D1 and D2) of the model predator in T1 and T2. The equations used for calculating D1 and D2 are as follows: PPD in T1 = $\sqrt{(235-D1)^2+65^2}$, PPD in T2 = $\sqrt{(235-D2)^2+25^2}$.

RESULTS

Eight of 48 cuttlefish ejected ink or fled from the arena during the trial in at least one of three trials. Also, one cuttlefish died during the experimental period after jetting out from its maintenance tank. We therefore could not collect the complete data set of the body patterns of these individuals and they were excluded from the analyses.

A total of 624 images of body patterns of *S. pharaonis* was extracted from the videos. Using these images, we classified the body patterns of *S. pharaonis* into seven major categories based on the chromatic components expressed on the mantle according to Hanlon and Messenger (1988): (1) "uniform light", (2) "disruptive", (3) "center circle", (4) "dark square", (5) "vertical stripe", (6) "all dark" and (7) "eyespots". (1) "Uniform light" is an overall light tone, lacking dark chromatic components (Fig. 4A). (2) "Disruptive" is characterized by transverse chromatic components such as White square, White mantle bar, Posterior transverse mantle line and Posterior mantle bar (Fig. 4B). (3) "Center circle" is characterized by the same chromatic components as "disruptive" but also contains Middle paired mantle spots and Central annulus (see Lee et al., 2010) (Fig. 4C). (4) "Dark square" is characterized by a set of dark chromatic components, which comprise a dark square component located in the same area as White square, Middle paired mantle spots and partial Posterior mantle bar (Fig. 4D). Anterior transverse mantle line and partial Anterior mantle bar are also expressed in this body pattern. (5) "Vertical stripe" is characterized by Median mantle stripe that transverses the lateral mantle vertically on one or both sides of the dorsal mantle. Anterior transverse mantle line and partial Anterior mantle bar are also expressed in this body pattern (Fig. 4E).

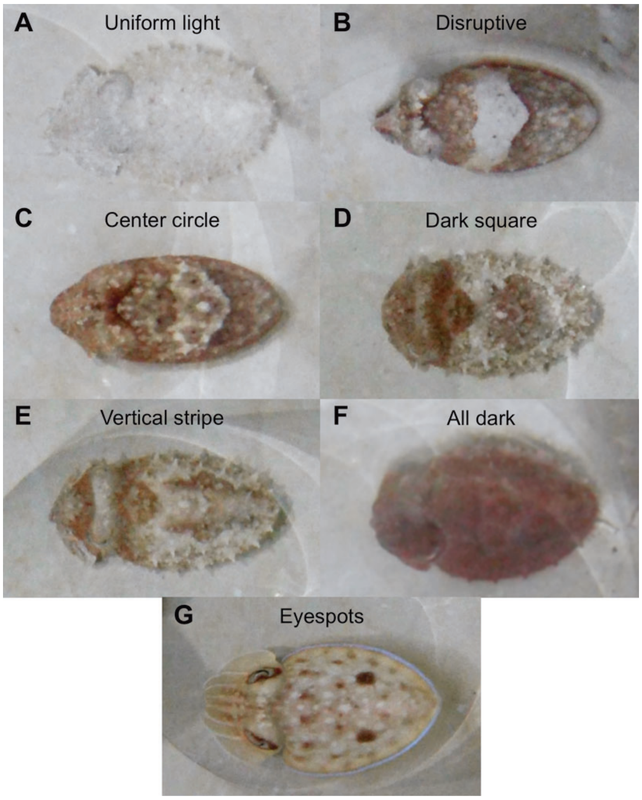

Fig. 4. Images of seven body patterns shown by *Sepia pharaonis* during the experiment. **(A)** "Uniform light". **(B)** "Disruptive". **(C)** "Center circle". **(D)** "Dark square". **(E)** "Vertical stripe". **(F)** "All dark". **(G)** "Eyespots".

(6) "All dark" is characterized by a uniformly darkened entire body (Fig. 4F). (7) "Eyespots" is characterized by a pair of dark eye-like spots on the posterior mantle (Fig. 4G). This pattern is mainly composed of Posterior paired mantle spots (see Lee et al., 2010). Eye ring and Mottle may also be expressed. We categorized 479 of the 624 images into one of the above seven major body patterns. The remaining 145 images could not be classified into any category because their patterns lacked chromatic components common to other images or because the number of observed cases was too small to classify into categories.

Body patterns expressed during control period

Sepia pharaonis mainly showed "uniform light", "disruptive" and "center circle", and rarely showed the other four body patterns, during the control period (Figs. 5 and 6). Cuttlefish did not change their body patterns during this period (Cochran's *Q* test; $P > 0.05$).

Body patterns expressed in T1

(1) The proportion of individuals that showed "uniform light" did not change among the five stages (Cochran's *Q* test; $Q = 6.286$, df = 4, $P = 0.179$) (Fig. 5A). (2) The proportion of individuals that showed "disruptive" significantly differed among the five stages ($Q = 17.74$, df = 4, $P = 0.001$). The proportion of individuals that showed "disruptive" decreased as PPD decreased (Fig. 5B). (3) The proportion of individuals that showed "center circle" did not differ significantly among the five stages ($Q = 9.474$, df = 4, $P = 0.050$) although it tended to decrease in frequency as PPD decreased (Fig. 5C). (4) The proportion of individuals that showed "dark square" differed among the five stages ($Q = 42.19$, df = 4, $P < 0.001$). "Dark square" was expressed only after the model predator was presented. The proportion of individuals that showed "dark square" increased as PPD decreased, especially when the model reached the closest PPD (Fig. 5D). (5) The proportion of individuals that showed "vertical stripe" did not differ significantly among the five stages ($Q = 8.952$, df = 4, $P = 0.062$). "Vertical stripe" was expressed only after the model predator was presented. The proportion of individuals that showed "vertical stripe" initially tended to increase as PPD decreased, but decreased rapidly when the model reached the closest PPD (Fig. 5E). (6) The proportion of individuals that showed "all dark" did not differ among the five stages ($Q = 6.880$, df = 4, $P = 0.142$). The proportion of individuals that showed "all dark" increased after the model predator was presented and was maintained while the model was approaching (Fig. 5F). (7) "Eyespots" was not expressed in T1 (Fig. 5G). The number of transitions of body patterns from stage 1 to stage 5 is shown in Table 1a. The body pattern of cuttlefish initially expressing "uniform light" tended to be stable, while those initially expressing "disruptive" or "center circle" tended to change their body patterns to "dark square".

Body patterns expressed in T2

(1) The proportion of individuals that showed "uniform light" did not change among the five stages ($Q = 6.435$, df = 4, $P = 0.169$). "Uniform light" was expressed at a relatively high rate from before the model predator was presented (Fig. 5A). (2) The proportion of individuals that showed "disruptive" did not differ among the five stages ($Q = 5.956$, df = 4, $P = 0.202$) although this pattern tended to decrease as the model approached (Fig. 5B). (3) The proportion of individuals that showed "center circle" did not differ among the five stages ($Q = 6.947$, df = 4, $P = 0.139$) although it tended to decrease as the model approached (Fig. 5C). (4) "Dark square" was rarely expressed (Fig. 5D), and the proportion of individuals that showed "dark square" did not differ among the five stages ($Q = 4.000$, df = 4, $P = 0.406$). (5) The proportion of individuals that showed "vertical stripe" differed among the five stages ($Q = 15.11$, df = 4, $P = 0.004$). "Vertical stripe" was not used before the model was presented. "Vertical stripe" was expressed immediately after the model was presented, and the proportion expressing it was very low during the model's approach (Fig. 5E). (6) The proportion of individuals that showed "all dark" differed among the five stages ($Q = 14.84$, df = 4, $P = 0.005$). "All dark" was used only after the predator was presented, and the proportion of individuals that showed "all dark" increased as the model approached but decreased at the closest PPD (Fig. 5F). (7) The proportion of individuals that showed "eyespots" differed among the five stages ($Q = 32.63$, df = 4, $P < 0.001$). "Eyespots" was displayed only when the model closely approached the cuttlefish (Fig. 5G). The number of transitions of body patterns from stage 1 to stage 5 is shown in Table 1b. There was no notable tendency in these numbers of transitions of body pattern.

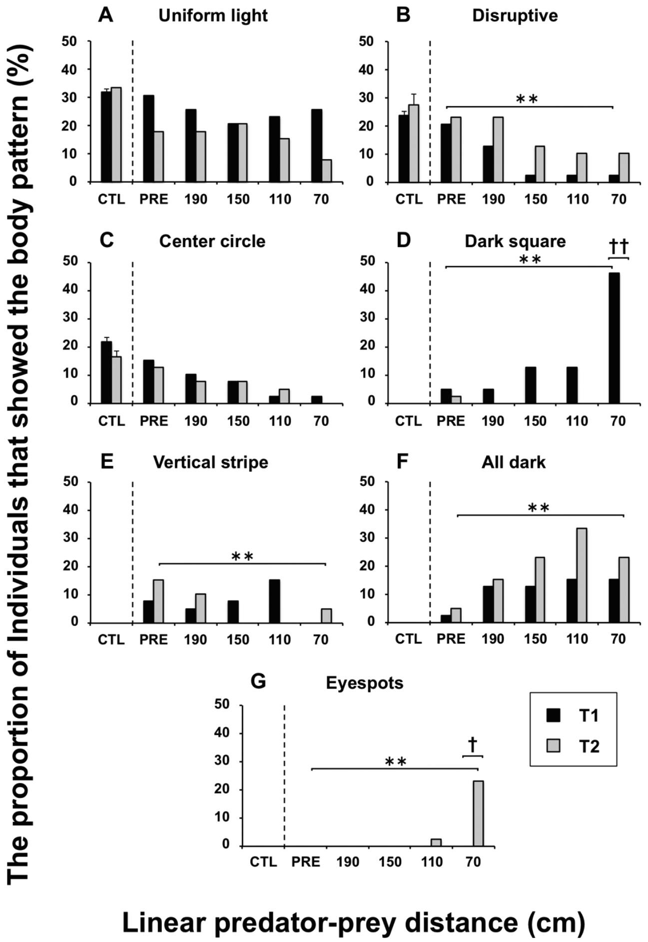

Fig. 5. Change of the proportion of individuals that showed each body pattern according to the decrease of the linear predator-prey distance in T1 and T2. Abbreviations: CTL, During control period; PRE, 3 s before the model predator began to move. See Fig. 2 for T1 and T2. Asterisks indicate significant differences among positions within a trajectory (* $P < 0.05$, ** $P < 0.01$; Cochran's Q test), and daggers indicate significant differences between the two trajectories († $P < 0.05$, †† $P < 0.01$; McNemar's test with Bonferroni correction).

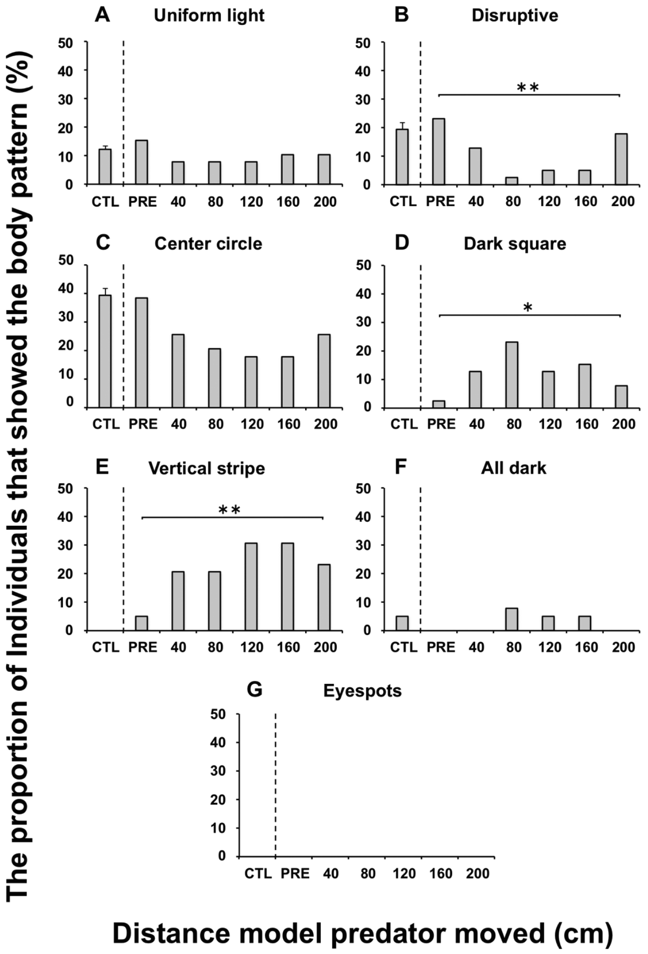

Fig. 6. Change of the proportion of individuals that showed each body pattern according to the movement of the model predator in T3. The linear PPD is shortest at 120 cm. The linear PPD at 80 cm and 160 cm would be similar. Likewise, the linear PPD at 40 cm and 200 cm would be similar. Abbreviations: CTL, During control period; PRE, 3 s before the model predator began to move. See Fig. 2 for T3. Asterisks indicate significant differences among positions (* $P < 0.05$, ** $P < 0.01$; Cochran's Q test).

Body patterns expressed in T3

(1) The proportion of individuals that showed "uniform light" did not change as the model moved ($Q = 5.540$, df = 5, $P = 0.354$). A low proportion of cuttlefish expressed "uniform light" before the model predator was presented (Fig. 6A). (2) The proportion of individuals that showed "disruptive" differed among the six stages ($Q = 17.11$, df = 5, $P = 0.004$). The proportion of individuals that showed "disruptive" patterning increased at the early and last stages of approaching, but decreased when the model made its closest approach to the cuttlefish (Fig. 6B). (3) The proportion of individuals that showed "center circle" did not differ significantly among the six stages ($Q = 9.040$, df = 5, $P = 0.107$). A relatively high proportion of cuttlefish expressed "center circle" before the model predator was presented, and this proportion tended to decrease as the model predator moved (Fig. 6C). (4) The proportion of individuals that showed "dark square" differed among the six stages ($Q = 12.42$, df = 5, $P = 0.030$). "Dark square" was used only after the model predator was presented. The proportion of individuals that showed "dark square" peaked in the middle of the trajectory, that is, the proportion increased as the model approached cuttlefish and decreased as the model receded from it (Fig. 6D). (5) The proportion of individuals that showed "vertical stripe" differed among the six stages ($Q = 15.70$, df = 5, $P = 0.007$). "Vertical stripe" was used only after the model predator was presented. The proportion of individuals that showed "vertical stripe" increased after the model began to move and remained relatively high while the model was moving (Fig. 6E). (6) Cuttlefish rarely expressed "all dark" (Fig. 6F) and (7) did not display "eyespots" (Fig. 6G). The numbers of transitions of body patterns from stage 1 to stage 3 and from stage 4 to stage 6 are shown in Table 1c and 1d, respectively. Cuttlefish initially expressing "disruptive" or "center circle" tended to change their body patterns to "dark square" or "vertical stripe" from stage 1 to stage 3 (Table 1c), while those initially expressing "center circle" tended to be stable,

and the cuttlefish initially expressing “vertical stripe” tended to change their body patterns back to “disruptive” or “center circle” from stage 4 to stage 6 (Table 1d).

Table 1. The numbers of transitions of body patterns expressed by *Sepia pharaonis* in each trajectory. For T1 (a) and T2 (b), transition from stage 1 to stage 5 is shown. For T3, transition from stage 1 to stage 3, that is, when the model predator is approaching the cuttlefish (c), and that from stage 4 to stage 6, that is, when the model predator is retreating from the cuttlefish (d), are shown. “ Others” indicate body patterns that were not able to be classified into any category.

(a)

Stage 5:	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Stage 1:								
(1) Uniform light	9			1		1		1
(2) Disruptive				6		2		
(3) Center circle				5				1
(4) Dark square				2				
(5) Vertical stripe	1		1					
(6) All dark						1		
(7) Eyespots								
(8) Others		1		3		3		1

(b)

Stage 5:	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Stage 1:								
(1) Uniform light	2	1					3	1
(2) Disruptive		1			1	1	2	4
(3) Center circle						3		2
(4) Dark square						1		
(5) Vertical stripe		1			1	2	1	1
(6) All dark	1							1
(7) Eyespots								
(8) Others		1				2	3	3

(c)

Stage 3:	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Stage 1:								
(1) Uniform light	3					1		2
(2) Disruptive		1	2	3	2			1
(3) Center circle			3	5	5	1		1
(4) Dark square			1					
(5) Vertical stripe				1	1			
(6) All dark								
(7) Eyespots								
(8) Others			2			1		3

(d)

Stage 6:	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Stage 4:								
(1) Uniform light	2	1						
(2) Disruptive	1	1						
(3) Center circle			6		1			
(4) Dark square			1	3	1			
(5) Vertical stripe		2	3		5			2
(6) All dark		1			1			
(7) Eyespots								
(8) Others	1	2			1			4

Comparison of body patterns between T1 and T2

(4) The proportion of individuals that showed “dark square” was significantly higher in T1 than in T2 when the linear PPD was 70 cm (McNemar’s test with Bonferroni correction, $P < 0.0001$) (Fig. 5D). (7) The proportion of individuals that showed “eyespots” was significantly higher in T2 than in T1 when the linear PPD was 70 cm (McNemar’s test with Bonferroni correction, $P = 0.0077$) (Fig. 5G). The other five body patterns showed no significant difference in proportion between T1 and T2 in any stages.

DISCUSSION

Sepia pharaonis expressed various body patterns in response to the model predator. We classified the body patterns of *S. pharaonis* into seven major categories according to the combination of chromatic components expressed in the dorsal mantle. The intensity of chromatic components differed both within and between cuttlefish during the experiment, but the combinations of characteristic chromatic components in each body pattern were consistent, which enabled us to distinguish the body patterns from each other. Four body patterns were identical to those classified as “uniform light”, “disruptive”, “all dark” and “eyespots” in many previous studies using *S. officinalis* (e.g., Adamo et al., 2006; Barbosa et al., 2007; Mäthger et al., 2007; Langridge et al., 2007; Langridge, 2009). Body patterns similar to two body patterns that we classified as “vertical stripe” and “center circle” have been reported in several previous studies (see Hanlon and Messenger, 1988; Lee et al., 2010). Previous studies have classified these body patterns as one form of “disruptive”. However, we classified these two body patterns and “disruptive” into different categories, as they are constructed from different sets of chromatic components and we can easily distinguish them from each other. The body pattern that we classified as “dark square” has not been reported in other species of *Sepia*. We named these three body patterns based on the characteristic feature in each body pattern.

Sepia pharaonis showed “uniform light”, “disruptive” and “center circle” during the control period. This suggests that these body patterns function as camouflage patterns, which was also suggested in many previous studies (e.g., Hanlon and Messenger, 1988; Lee et al., 2010). Although “uniform light” is assumed to function as camouflage on a uniform background such as the substrate used in this study, “disruptive” and “center circle” are considered to be effective camouflage patterns only on visually complex substrates that contain light and dark patches (Hanlon, 2007; Barbosa et al., 2008). Recently, however, Ulmer et al. (2013) demonstrated that *S. officinalis* shows “disruptive” in a uniform environment when there are high-contrast elements even if they constitute only a small proportion of its visual field. The experimental tank used in our study was basically uniform white, but there were a few small organisms attached to the bottom and the wall of the tank. The organisms were red and brown colored, and therefore they constructed a high-contrast pattern with the bottom and the wall of the tank. It is possible that *S. pharaonis* selectively responded to the attached organisms inside the tank and used “disruptive” and “center circle” as camouflage patterns.

During the control period, cuttlefish did not change their body patterns. This indicates that cuttlefish were sufficiently acclimated to the experimental condition before the model predator was presented. Cuttlefish temporarily changed body patterns in response to the sudden removal of the opaque board, but all cuttlefish recovered their body pattern within 30 s. Therefore, we are confident that the transition of body pattern during the trial was induced by the movement of the model predator. However, there is a possibility that cuttlefish responded to the moving model without recognizing it as a real predator, but simply recognized it as an unfamiliar object. Nevertheless, many cuttlefish in our experiment showed secondary defenses, such as deimatic patterns, jetting and inking, supporting the assumption that the model predator worked as a threatening stimulus for cuttlefish, and it is reasonable to assume that the cuttlefish used primary and secondary defenses to avoid this potentially threatening object.

We predicted that *S. pharaonis* would change its body pattern depending on the predation risk that is associated with PPD and the trajectory of the approaching predator. In our study, predation risk can be conceived 1) as very low before presentation of the model predator, 2) to increase as PPD decreases in T1 and T2, and 3) to increase in the anterior half of trajectory and decrease in the posterior half of the trajectory in T3. Additionally, even when the linear PPD is the same, predation risk would be higher in T2 than T1, because the model in T2 approached cuttlefish more directly. As we predicted, the results indicated that cuttlefish changed its body pattern according to PPD, and also indicated that the trajectory of the approaching model affected the body pattern of cuttlefish even when the linear PPD was the same. “Eyespots”, which is assumed to function as secondary defense (Langridge, 2009; Staudinger et al., 2013), was expressed only in the situation expected to have high predation risk, that is, when linear PPD was short in T2. On the other hand, “uniform light”, “disruptive” and “center circle”, which are supposed to function as camouflage, tended to be used in the situations expected to be low predation risk, that is, before the model was presented and when PPD was long. On the other hand, “dark square”, “vertical stripe” and “all dark” tended to be used in the situations when the predation risk was expected to be intermediate, which makes it difficult to determine whether these body patterns function as primary or secondary defense.

It is possible that factors other than predation risk also affect the expression of body patterns. *Sepia pharaonis* may change its camouflage patterns according to PPD and the trajectory of a nearby predator because these would affect how cuttlefish appear to a predator. This PPD-dependent appearance is attributed to limitations of the visual system of the predator, such as spatial acuity and contrast sensitivity (Endler, 1992; Stevens, 2007). Similarly, the trajectory-dependent appearance is attributed to the angle of viewing by the predator. The predator in T1 and T3 would see cuttlefish from above, whereas the predator in T2 would see cuttlefish from the side. In our study, “dark square” and “vertical stripe” were frequently expressed in T1 and T3, respectively. The observed number of transitions of body patterns in T1 indicates that the cuttlefish initially expressing “disruptive” or “center circle” changed their body patterns mainly to “dark square” (Table 1). The observed number of transitions of body patterns in the anterior half of T3 indicates that the cuttlefish initially expressing “disruptive” or “center circle” tended to change their body patterns to “dark square” or “vertical stripe” (Table 1), and that in the posterior half of T3 indicates that cuttlefish expressing “vertical stripe” changed their body patterns back to “disruptive” or “center circle” (Table 1). From these results, we suggest that “vertical stripe” and “dark square” may function as camouflage patterns that take the place of “disruptive” and “center circle” when the latter two body patterns become less effective, that is, when predators come to view the cuttlefish from above. However, there is also a possibility that mechanoreceptive cues induced “vertical stripe” and “dark square”. The transparent arena was open-topped and 60 cm in height, and the model predator in T1 and T3 moved 70 cm above the bottom of the tank, whereas the model in T2 moved 30 cm above the bottom of the tank. Therefore, it is possible that mechanoreceptive cues from the moving model in T1 and T3 were transmitted from the top of the arena through water to the cuttlefish inside the arena when the model was moving very close to the cuttlefish.

“Uniform light” and “disruptive” were both expressed from before the model predator was presented, which supports the notion that these two body patterns are used for camouflage, as mentioned above. However, the proportion of individuals that showed these two body patterns changed differently in response to the approach of the model predator. The proportion of individuals that used “uniform light” changed only slightly depending on the movement of the predator, whereas the proportion that used “disruptive” was significantly affected by the movement of the predator. The observed number of transitions of body patterns indicates that the cuttlefish initially expressing “uniform light” changed their body pattern less frequently compared to the cuttlefish initially expressing “disruptive” (Table 1). Accordingly, the eventual body patterns seemed to differ between the cuttlefish initially expressing “uniform light” and “disruptive” (Table 1). This result suggests that *S. pharaonis* changes its antipredator response according to its initial camouflage pattern.

The function of “all dark” is also unclear from our results. This body pattern was used most frequently when predation risk was assumed to be intermediate. One of the possible functions of “all dark” is masquerade, that is, *S. pharaonis* may mimic inedible objects such as a clump of seaweeds (see Buresch et al., 2011). Alternatively, “all dark” may function as a secondary defense, as several previous studies have suggested for *S. officinalis* (e.g., Adamo et al., 2006; Langridge, 2009; Staudinger et al., 2013).

We note that *S. pharaonis* in T1 and T2 showed “uniform light” most frequently during the control period, whereas *S. pharaonis* in T3 showed “center circle” most frequently during this period. We presume that this was because the visual environment during the acclimation period differed between the experiments. The relative position between the opaque board and the cuttlefish differed, that is, the cuttlefish in T1 and T2 would see the board located at the opposite side of the tank, whereas the cuttlefish in T3 would see the board located at a relatively close distance compared to T1 and T2 during the acclimation period. This difference may have affected the camouflage patterns of cuttlefish during this period.

It is noteworthy that Langridge (2009) showed that *S. officinalis* does not display "eyespots" in response to large teleosts, which are the model predator of our stimulus. Recently, however, Staudinger et al. (2013) demonstrated that *S. officinalis* displays "eyespots" in reponse to large teleosts. Staudinger et al. (2013) suggested that this discrepancy could be attributed to the difference in experimental design between the studies. Nonetheless, the experimental design of our study was more similar to that of Langridge (2009) than that of Staudinger et al. (2013). Both Langridge (2009) and the present study used a transparent arena to separate predators from cuttlefish, whereas Staudinger et al. (2013) did not use arenas in order to better simulate natural predator-prey encounters. Transparent arenas can restrict the movement of cuttlefish, which helps researchers to observe their behavior, and also prevents cuttlefish from being eaten by predators, which may raise ethical issues. On the other hand, a transparent arena may affect the anti-predator response of cuttlefish because it would restrict the movement of the cuttlefish and make tactics such as fleeing ineffective. It may also disrupt information such as chemosensory and mechanoreceptive cues from predators (Boal and Golden, 1999; Komak et al., 2005). Further experiments are needed to clarify the factors that induce the "eyespots" response.

It should be noted that we presented cuttlefish only visual cues. Although cephalopods generally have excellent visual acuity and large optic lobes (Mather and Kuba, 2013) and their body patterns are strongly affected by visual cues (Williamson and Chrachri, 2004), cephalopods are also suggested to use modalities other than visual cues (i.e., chemoreceptive and mechanoreceptive cues) for avoiding predation (see Boal and Golden, 1999; Komak et al., 2005). Therefore, *S. pharaonis* may show responses different from the present observations if other cues are available. Further studies are necessary to examine the effects of chemoreceptive and mechanoreceptive cues on the body pattern changes of *S. pharaonis*.

In the present study, we demonstrated that the distance from a model predator and the trajectory of the model predator affect the body pattern of *S. pharaonis*. These results suggest that *S. pharaonis* is able to evaluate predation risk and the view-point of a predator based on the predator's movement and change its body pattern accordingly. Previous studies demonstrated that *S. officinalis* shows various anti-predator responses according to the species of predator (e.g., Staudinger et al., 2013), but those studies did not clarify the effect of specific factors that may affect the response of *S. officinalis* (e.g., appearance, movement, chemical cues and mechanosensory cues). We used a model predator to precisely control the visual cues of the movement of the predator stimulus to enable us to examine the effects of distance from a predator and its trajectory. Further studies using a model predator are expected to help to clarify the effects of other factors associated with the movement of the predator (e.g., speed of approach and sudden change in trajectory) on body patterns shown by the cuttlefish, which will lead to deeper understanding of their functions.

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