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Behavioral Laterality and Morphological Asymmetry in the Cuttlefish, *Sepia lycidas*

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Behavioral laterality is widely found among vertebrates, but has been little studied in aquatic invertebrates. We examined behavioral laterality in attacks on prey shrimp by the cuttlefish, *Sepia lycidas*, and correlated this to their morphological asymmetry. Behavioral tests in the laboratory revealed significant individual bias for turning either clockwise or counterclockwise toward prey, suggesting behavioral dimorphism in foraging behavior. Morphological bias was examined by measuring the curvature of the cuttlebone; in some the cuttlebone was convex to the right (righty), while in others, the cuttlebone was convex to the left (lefty). The frequency distributions of an index of cuttlebone asymmetry were bimodal, indicating that populations were composed of two types of individuals: “righty” and “lefty.” Moreover, an individual’s laterality in foraging behavior corresponded with the asymmetry of its cuttlebone, with righty individuals tending to turn counterclockwise and lefty ones in the opposite direction. These results indicate that cuttlefish exhibit behavioral dimorphism and morphological antisymmetry in natural populations. The presence of two types of lateral morph in cuttlefish provides new information on the relationship between antisymmetric morphologies and the evolution of individual laterality in behavioral responses in cephalopods. The implications of these findings for the interpretation of ecological meaning and maintenance mechanisms of laterality in cuttlefish are also discussed.

Key words: cuttlebone, lefty, righty, clockwise turn, counterclockwise turn, antisymmetry

INTRODUCTION

Lateral asymmetry is found in various vertebrate taxa (Rogers, 2002). Behavioral laterality is believed to be due to the structural and functional superiority of one side of the body, especially the brain, over the other (Vallortigara and Rogers, 2005), although relatively few studies have addressed cerebral bias as a morphological asymmetry. Recently, however, it has been revealed that many fish exhibit lateral dimorphism that correlates with behavioral laterality (Hori, 1993; Seki et al., 2000; Nakajima et al., 2007; Takeuchi and Hori, 2008; Yasugi and Hori, 2011).

Lateral asymmetry can be generally divided into three categories based on the shape of the distribution of the lateral asymmetry: fluctuating asymmetry (FA) with a unimodal and symmetrical distribution; directional asymmetry (DA) with a unimodal distribution shifted from symmetry; and antisymmetry (AS) with a bimodal distribution (Palmer and Strobeck, 1986). Hori (1993) first detailed antisymmetric trophic morphs and related behavioral laterality in the scale-eating cichlid fish of Lake Tanganyika. In these species, the mouth opens either rightward or leftward, owing to asymmetrical joints of the mandibles to the suspensorium (Liem

and Stewart, 1976; Hori, 1991), and attack direction toward prey corresponds with mouth asymmetry in these fish (Hori, 1993). This dimorphism appears to be maintained by frequency-dependent natural selection, with advantages to rare morphs under natural conditions (Hori, 1993). Such laterality has now been found in other fishes from a variety of trophic levels, and every population studied has been composed of two types of individuals; a right-dominant type (called righties) and a left-dominant type (lefties) (Mboko et al., 1998; Seki et al., 2000; Nakajima et al., 2007; Takeuchi and Hori, 2008; Yasugi and Hori, 2011). Recent works have proved that this laterality is a genetic trait (Hori et al., 2007; Seki et al., 2000; Stewart and Albertson, 2010; Hata et al., 2012; Hata and Hori, 2012).

Given that laterality has been found over a broad range of vertebrate taxa, it would be reasonable to expect that some, or indeed many, invertebrate taxa may have this trait. However, aside from studies of snails, fiddler crabs, and some insects that exhibit clear-cut lateral asymmetry in their external morphology, only a few reports have explored behavioral laterality in invertebrates, with primary focus on crustaceans (Hamilton et al., 1976; Takeuchi et al., 2008; Tobo et al., 2012) and cephalopods (Alves et al., 2007; Byrne et al., 2002, 2004).

In some crustaceans, morphological antisymmetry is prominent and related behavioral lateralization has been studied (Takeuchi et al., 2008; Tobo et al., 2012). The abdominal antisymmetry of atyid shrimps is related with rightward or leftward jumping direction for escape, and such

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antisymmetry seems to be a genetic character (Takeuchi et al., 2008). Tobo et al. (2012) revealed lateral antisymmetry in the carapaces of crayfish, corresponding to the direction of their backward-jumping escape behavior. These authors suggested that the lateral dimorphism of these crustaceans was also maintained by frequency-dependent natural selection through the interaction between predator and prey populations.

In cephalopods, *Octopus vulgaris* utilizes monocular vision almost exclusively when staring at an object and approaching prey and shows antisymmetrical distributions of lateralization at the population level (Byrne et al., 2002, 2004). Another group of cephalopods, cuttlefish, also shows a lateralized behavior in approaching prey and other situations. When juvenile of the European common cuttlefish, *Sepia officinalis*, attempt to attack a crab, they avoid the claws by swimming above the crab and turning around rightward or leftward to approach it from behind (Wells, 1962; Hanlon and Messenger, 1996), although individual bias of the turning direction has not been documented for this behavior. More specifically, however, when adult *S. officinalis* are allowed to freely enter two arms of a T-maze, some individuals tend to turn right whereas others turn left (Karson et al., 2003; Alves et al., 2007). Though morphological asymmetry has not previously been known in cephalopods, it seems to be possible in cuttlefish to examine morphological asymmetry, if ever, using their internal hard shell, cuttlebone.

The purpose of the present study is to demonstrate that cuttlefish individuals have a left or right behavioral bias during their capture of a prey shrimp and to correlate this behavioral bias with morphological asymmetry based on the curvature of the cuttlebone. We used the kisslip cuttlefish, *Sepia lycidas* Gray 1849, a common and a large-sized (38 cm adult mantle size) univoltine cuttlefish found in littoral areas around Japan and Southeast Asia (Carpenter and Niem, 1998), which is available for rearing under laboratory conditions.

MATERIALS AND METHODS

Subjects and maintenance

In laboratory tests, we detected lateral bias in the attacking behavior of young *S. lycidas* in their positioning toward a target prey shrimp. Young cuttlefish were used due to their ease of handling in the aquarium. The morphological asymmetry of all subjects was examined by measuring the curvature of their cuttlebone after the behavioral test.

Behavioral tests were conducted in the aquarium of the Ashizuri Marine Museum of Tosashimizu City, Kochi Prefecture, Japan, from August to November in 2002, 2006, 2007, and 2008. To acquire young cuttlefish, mature adult *S. lycidas* were collected from a fixed net at the Iburi coast in Tosashimizu City every summer and were bred in the aquarium. Every pair of parents was used for breeding in 2002 and 2008, and every two pairs were used in 2006 and 2007.

For breeding, every pair was kept in a plastic basket (50 cm in diameter and 40 cm in height), which was suspended in a large tank (1 m × 2 m × 0.5 m) with circulating filtered sea water of 30 cm depth and fine sand 3 cm thick. When oviposition was completed in the basket, the parents were transferred into other tanks. At 23 to 25°C water temperature, the eggs required 25 to 30 days to hatch, and the hatchlings (dorsal mantle length, DML: about 7 to 8 mm) began to forage immediately. They were fed on live mysid shrimp (*Neomysis japonica* Nakazawa 1910) until they grew to a size of 1

cm DML in about two weeks, and were then fed on palaemonid shrimp, *Palaemon pacificus* (Stimpson 1860), of suitable sizes until they grew to 3 to 5 cm DML in about one month. These prey shrimp were collected from the shore around the institute. The young *S. lycidas* were then used for behavioral tests and/or morphological measurements; in total 35 individuals were numbered and subjected to the behavioral tests and subsequent measurement (6, 12, 11, and 6 young in 2002, 2006, 2007, and 2008, respectively). An additional 30 specimens were used only for morphological measurement. We were unable to identify the sex of the young cuttlefish.

Behavioral tests

Foraging behavior was observed in an arena (a square glass tank, 370 × 400 × 100 mm depth) using a digital video camera (Sony DCR-PC120, 1.5 megapixels) each year. The arena was filled with filtered seawater of 5 cm depth, and the bottom was covered with a 20 mm layer of fine sand to allow the subject individuals to hide themselves. The wall of the arena was covered by black cloth. In 2002 and 2008, six arenas were prepared for six individuals, and in 2006 and 2007, the six arenas were used such that on one day six individuals (Nos. 1 to 6) and on the next day another six individuals (Nos. 7 to 12) were observed. We used young (body length 2 to 3 cm) palaemonid shrimp, *P. pacificus*, as prey in the behavioral tests.

In each trial, a shrimp was attached to a needle with a long shaft and was manually presented to the subject cuttlefish so the subject and shrimp were positioned face to face and at least 10 cm apart from each other. The behavior of the cuttlefish was then recorded. To record the sequence of attacking behavior, the video camera was set at 50 cm height over the arena. Thirty attacks toward the prey were recorded for each subject cuttlefish. Two hours before the first trial on each day, a palaemonid shrimp was provided to each cuttlefish to calm them in the observation arena. The maximum recording time was 5 min for each observation. If the subject did not show any attack behavior within 5 min, the test was terminated on that day. Within a day, each individual was used once every two hours. The number of trials for each individual in one day varied depending on its appetite and was usually between four and six.

When approaching a relatively large prey shrimp and being allowed to move freely in any direction, the young cuttlefish turned widely to take a position to the side or sometimes diagonally from behind the prey before seizing it (Fig. 1). We judged whether each subject turned clockwise or counterclockwise from the direction of the cuttlefish 1 sec after starting its move toward the shrimp (rightward or leftward) by playing back the video images, and a chi-square test was applied to examine the leftward and rightward bias of each individual.

To ascertain the type of asymmetry in the hunting behavior of *S. lycidas* as a whole, we analyzed the pattern of an index of behavioral laterality (IBL) (Bisazza et al., 1998), which is expressed by the formula

$$[(T_R - T_L) / (T_R + T_L)] \times 100,$$

where T_R is the number of counterclockwise turns (rightward turns) and T_L is the number of clockwise turns (leftward turns).

The behavioral laterality between left and right biased of an animal may discriminate as nonlateralized behavior or random behavior with a unimodal and symmetrical distribution, laterality at population level with a normal distribution depart from symmetrical, where majority of individuals are lateralized in the same direction and causing a population bias to either the right or the left, and individual laterality with an antisymmetry distribution, where majority of individuals are also lateralized but there are approximately equal numbers of right and left biased individuals in the population (Denenberg, 1981; Lehman, 1981; Vallortigara and Rogers, 2005).

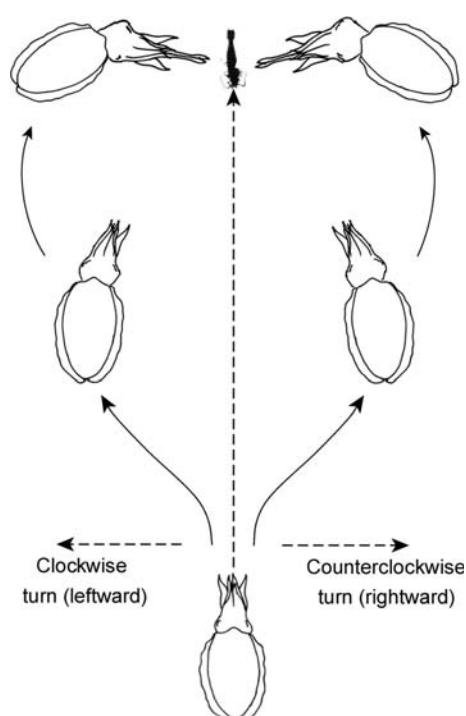


Fig. 1. Diagram showing lateralized behavior in prey capture in a behavioral test (upper view) and the criterion for judging turning direction. When attacking a prey shrimp, the young cuttlefish turns and swims to take a position from the side or diagonally behind the shrimp. The direction of movement (rightward or leftward) 1 sec after starting toward the target was used to define the turning direction (clockwise or counterclockwise).

To examine the frequency distribution of the IBL for all individuals examined, a test of model fit for nonlateralized behavior or random behavior (called FA model following terminology in morphological asymmetry), behavioral laterality at population level (DA), and individual laterality (AS) models was applied using the R statistical package (R version 2.11.1). In the FA model, the distribution is normal with means $\mu_1 = \mu_2 = 0$ and standard deviations $\sigma_1 = \sigma_2$ of the data. In the DA model, the distribution is normal with means μ_1, μ_2 ($\neq 0$) and standard deviations σ_1, σ_2 of the data, and in the AS model, the distribution is bimodal with means $(+\mu_1, -\mu_2)$ (the mean, $+\mu_1$ and $-\mu_2$ are related with the frequency distributions of the rightward and leftward turns, respectively) and standard deviations $\sigma_1 = \sigma_2$ of the data. In these three models the approximate values for parameters were calculated by the most likelihood methods. We calculated Akaike's information criteria (AIC) for use in comparing these three models. The best-fitting model was selected based on the lowest AICs of the three models (using a difference of greater than 1.0 as an empirical criterion). Such three models analysis was used previously by Yasugi and Hori (2011) to determine the distribution patterns of index of asymmetry in the height of mandible posterior ends of largemouth bass and in the length of dentary of *Rhinogobius goby*.

Measurement and statistics of morphological asymmetry in cuttlefish

Thirty-five young cuttlefish were observed for the behavioral test and an additional 30 were used for the measurement of the morphological asymmetry of their cuttlebones. All specimens were paralyzed and fixed with chilled seawater, and their cuttlebones were separated. As well as these young specimens, 72 juvenile and

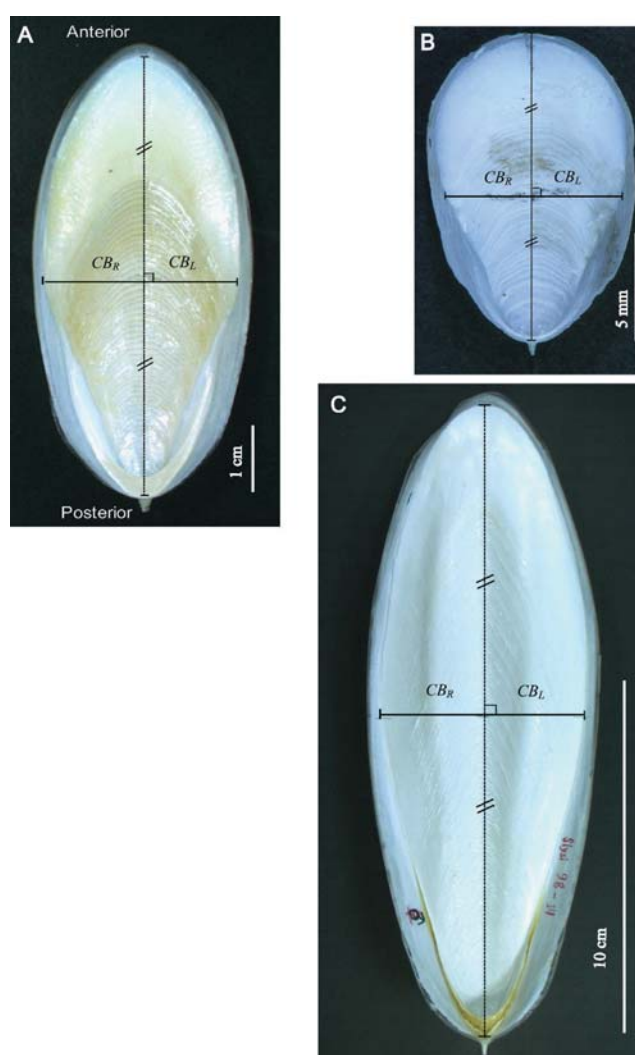


Fig. 2. Datum points for the measurement of asymmetry in the cuttlebones of young (A), juvenile (B), and adult (C) *S. lycidas* (ventral view). R and L are the lengths of the right and left sides, respectively (at the medial region), from the longitudinal intersection line between the two ends of the cuttlebone.

67 adult cuttlefish were also used for the same purpose. The juveniles were obtained from our breeding, and the adults were purchased from fishermen around Tosashimizu in 2010 and 2011. The rearing, behavioral tests and preparation of morphological specimens were conducted in accordance with the Guidelines for Animal Experimentation, Kyoto University.

To quantify the asymmetry of the cuttlebones, we measured the curvature of each cuttlebone as follows. After the length of the cuttlebone was measured using vernier calipers, the ventral view of each cuttlebone was photographed by a digital microscope (VHX-100, Keyence Co., Japan) while maintaining the horizontal level of each specimen. The anterior and posterior ends of the cuttlebone were designated as the tip of the median sulcus (anterior) and the base of the spine (posterior), respectively (Fig. 2). At the middle of the longitudinal line between the two ends, a perpendicular line was drawn on the monitor of the digital microscope, and the length between the right (CB_R) and left (CB_L) sides of the cuttlebone from the intersection were measured. The measurements were repeated three times for each cuttlebone, independently mounting it on the stage each time, and the median value was adopted for the analy-

sis, because measurement of asymmetry produced some extreme values (e.g. $IA_1 = -9.125$, $IA_2 = -7.940$, $IA_3 = -8.303$). The curvature of each cuttlebone was quantified using the index of asymmetry (IA) (Palmer and Strobeck, 1986) as follows:

$$IA = 2\{(CB_R - CB_L) / (CB_R + CB_L)\} \times 100.$$

The laterality of each cuttlefish was defined such that an individual with a positive IA value was a 'righty' (i.e., the cuttlebone was convex to the right implying that the right side of the body was more developed than the left side), and an individual with a negative IA value was a 'lefty.'

To examine the frequency distribution of the IA of cuttlebones observed in the morphological examination, a test of model fit for the FA, DA, and AS models was applied using the R statistical package (following Yasugi and Hori, 2011). To determine the deviation of distribution of each IA from normal, a Shapiro-Wilk test was also applied. To analyze the relationship between the behavioral laterality and morphological asymmetry of cuttlefish, a generalized linear model (GLM) was applied with behavioral laterality,

$$\arctan [(T_R - T_L) / (T_R + T_L)],$$

as the dependent variable, and morphological asymmetry,

$$\arctan 2[(CB_R - CB_L) / (CB_R + CB_L)],$$

and difference in years as independent variables. GLM analysis was conducted using JMP version 5 (SAS Institute Inc., Cary, NC, USA). Pearson's test (Sokal and Rohlf, 1995) was applied to examine the relationship of the degree of IA of cuttlebone and that of the growth of cuttlefish, and also to determine the relationship between the degree of IBL and IA in each individual cuttlefish.

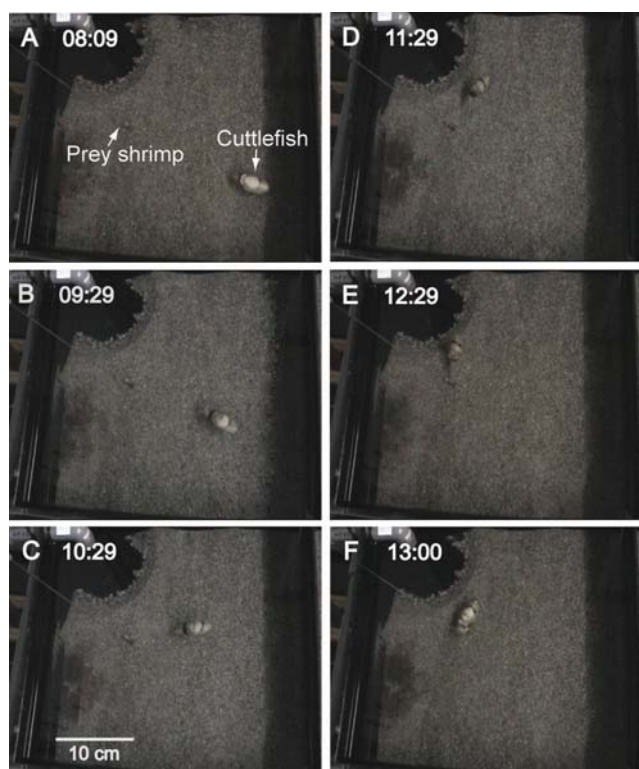


Fig. 3. An example of the attacking sequence of a young *S. lycidas* from approach to seizure of the prey shrimp. The time indicated is second: (namely, third in 1/60 seconds) from the start of the observation record. It took five seconds from the cuttlefish starting to move toward the prey to seizing it. See text for the details of the sequence of cuttlefish behavior.

RESULTS

Behavioral laterality of cuttlefish

In general the attacking behavior of a young *S. lycidas* on a palaemonid proceeded as described below (see Fig. 3). As shown in Fig. 3A, when the cuttlefish noticed the shrimp being presented face to face, it began to swim slowly and directly toward the shrimp. When reaching a distance of two to three times its body length from the prey, the cuttlefish began to turn rightward (Fig. 3B) or leftward. The cuttlefish then continued to turn while keeping its head toward the prey (watching the target with both eyes), raising its arms up and eventually raising the body upward (Fig. 3C). It then took a position to the side, or sometimes diagonally, behind the prey (Fig. 3D), stretched its tentacles downward and held the abdomen of the prey (Fig. 3E), and engulfed the prey by jumping on it (Fig. 3F). This process took three to four seconds from the start of turning to the capture of the target.

Every cuttlefish showed a distinct preference for either clockwise or counterclockwise turning behavior on its approach to the prey in each year (see Supplementary Table S1). As shown below in the results of the GLM analysis, no significant difference was found between years, and thus the results for the four years were pooled for analysis. Of the 35 young cuttlefish examined, 14 exhibited significant bias for clockwise (leftward) and 14 for counterclockwise (rightward) turning in prey-catching behavior (chi-square test, $P < 0.05$). The frequency distributions of the IBL significantly differed from normal (Shapiro-Wilk normality test, $W = 0.859$, $P < 0.01$) and best fitted to the models of bimodal distribution (Fig. 4).

Morphological asymmetry of cuttlefish

Morphological measurements of young (cuttlebone length, mean \pm SD: 72.84 ± 23.18 mm) *S. lycidas* showed that they comprised two types of individuals, and could be classified as either lefties (IA, mean \pm SD: -4.17 ± 2.43 , $n = 32$) or righties (5.84 ± 2.51 , $n = 33$) (Fig. 5A). The frequency distribution of the IA in young *S. lycidas* differed significantly from a normal curve (Shapiro-Wilk normality test: $W = 0.948$, $P < 0.01$) and defined as antisymmetry.

Morphological asymmetry in *S. lycidas* was also found both in juveniles (cuttlebone length, mean \pm SD: $16.33 \pm$

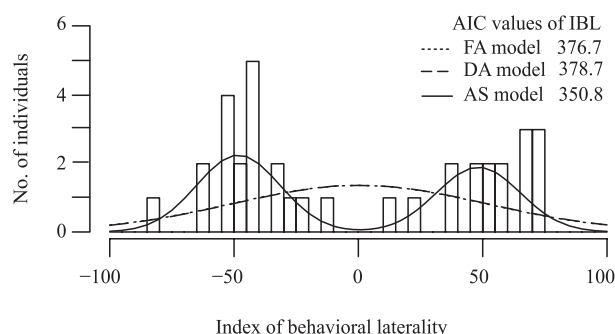


Fig. 4. Frequency distribution of the index of behavioral laterality (IBL) of young *S. lycidas* in the behavioral tests in 2002, 2006, 2007, and 2008 ($n = 35$).

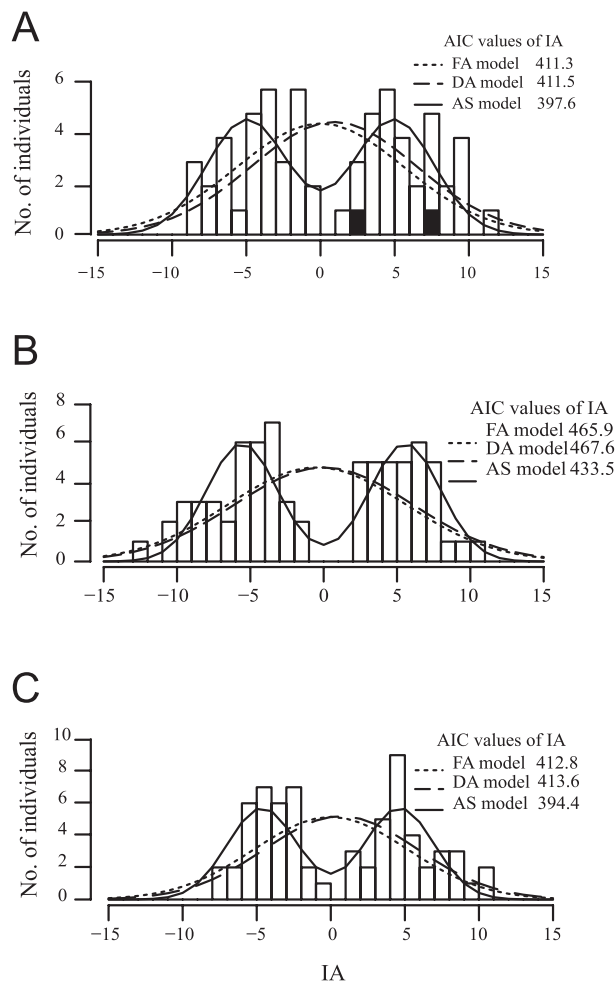


Fig. 5. Histogram of the index of asymmetry (IA) of cuttlebones of young ($n = 65$) (A), juvenile ($n = 72$) (B), and adult ($n = 67$) (C) *S. lycidas*. (A) Dark columns represent the cuttlefish that tended to turn leftward (clockwise) in behavioral tests but were morphologically righty individuals.

Table 1. Results of GLM analysis on the behavioral laterality of *S. lycidas*.

Variable	Df	Coefficient	Sum of square	F	P
Year	3		0.258	1.120	0.356
Morphological laterality	1	7.151	4.423	57.569	< 0.001

5.60 mm) with either lefties (IA, mean \pm SD: -5.71 ± 2.69 , $n = 38$) and righties (5.42 ± 2.08 , $n = 34$) (Fig. 5B) and in adults (cuttlebone length, mean \pm SD: 192.22 ± 23.24 mm) in lefties (IA, mean \pm SD: -4.04 ± 1.77 , $n = 33$) and righties (5.29 ± 2.56 , $n = 34$) (Fig. 5C). The frequency distributions of the IA in both juveniles and adults were also significantly different from normal (Shapiro-Wilk normality test: $W = 0.934$, $P < 0.01$ and $W = 0.936$, $P < 0.01$, respectively) and defined as antisymmetry.

The degree of IA of cuttlebone was not correlated with the cuttlefish growth either in righty or lefty individuals (for righties, Pearson's test: $r = 0.045$, $n = 33$, $P > 0.05$, in young, $r = 0.032$, $n = 34$, $P > 0.05$, in juveniles, and $r =$

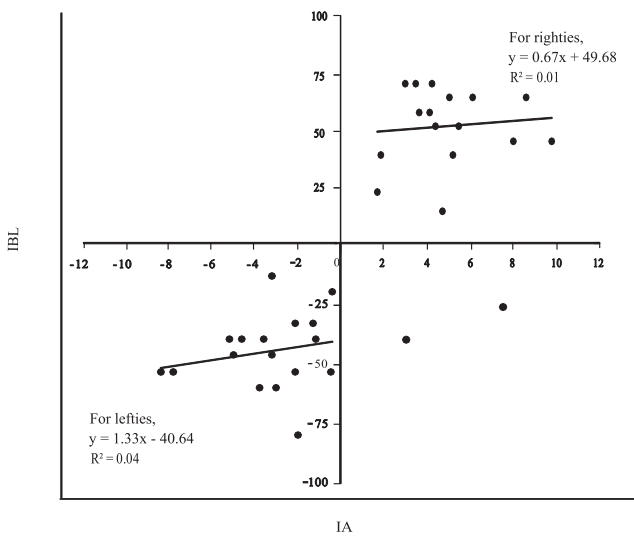


Fig. 6. Relationship between the magnitude of the index of behavioral laterality (IBL) and that of the index of asymmetry (IA) of the cuttlebones of *S. lycidas* of each morph. The correlation is not significant (Pearson's test: for righties, $t = 0.378$, $P > 0.05$; for lefties, $t = 0.760$, $P > 0.05$).

0.046, $n = 34$, $P > 0.05$, in adults; for lefties, Pearson's test: $r = 0.001$, $n = 32$, $P > 0.05$, in young, $r = 0.048$, $n = 38$, $P > 0.05$, in juveniles, and $r = -0.005$, $n = 34$, $P > 0.05$, in adults; after sequential Bonferroni correction).

Relationship between morphological asymmetry and behavioral laterality in cuttlefish

The results of GLM analysis indicated that morphological asymmetry has a positively significant effect on the behavioral laterality of cuttlefish (Table 1), indicating that righties tended to turn counterclockwise rather than clockwise, whereas lefties showed the opposite tendency in their attacking behavior. Differences in year exerted no significant effect on laterality. We found no significant correlation between the magnitude of the IBL during turning and that of the IA of the cuttlebone (Fig. 6) (Pearson's test: $t = 0.378$, $P > 0.05$, for righties; $t = 0.760$, $P > 0.05$, for lefties; after sequential Bonferroni correction).

DISCUSSION

In their approach to prey shrimp, most of the *S. lycidas* individual showed a bias toward turning either rightward or leftward. Moreover, we found a bimodal distribution pattern in the index of behavioral laterality as a whole for the individuals tested, with a few exceptional individuals turning both rightward and leftward evenly. Thus, it can be said that the cuttlefish shows behavioral dimorphism in its hunting of shrimp. Behavioral dimorphism has also been found in tests examining cuttlefish behavior in turning into a T-maze (Karson et al., 2003; Alves et al., 2007) and in eye use by octopuses watching subjects (Byrne et al., 2002, 2004). Our study confirms that cephalopods exhibit laterality in their foraging behavior.

The results of the present study also reveal morphological asymmetry in juvenile, young, and adult *S. lycidas* in the curvatures of their cuttlebones. The frequency distribution of

the IA of cuttlebones was categorized as antisymmetry rather than as fluctuating asymmetry or directional asymmetry. Thus, the population of *S. lycidas* comprised two morph types: righties and lefties. In righties the cuttlebone was convex to the right, i.e., the right side of the body was more developed than the left, and in lefties, the left side was more developed than the right. Moreover, patterns of morphological asymmetry found in juvenile, young and adult is consistent, though their body size is largely different. Therefore, we can predict that morphological asymmetry is not an acquired character but rather belongs to the cuttlefish.

We found a strong correspondence between the bias in turning direction during prey capture and the asymmetry of the cuttlebone. Righties and lefties showed significantly more counterclockwise and clockwise turning behavior, respectively, in their prey approaches. Two exceptional individuals (righties) showed significantly more counterclockwise turns than expected from the asymmetry direction of their cuttlebones. Although turning direction was affected by cuttlebone asymmetry, the direction of all attacks was not always fixed with the asymmetry of the cuttlebone. Furthermore, we found that the degree of behavioral laterality showed no relation to the degree of asymmetry of the cuttlebone. This suggests that the curvature itself is not the cause of behavioral laterality, but that some other internal factors may also be involved. Takeuchi et al. (2008) presented a similar result, showing that the abdominal asymmetry of shrimp was related with their behavioral laterality during evasive response, but that the degree of the behavioral laterality of shrimp was not affected by the degree of morphological asymmetry of the abdomen. This is also supported by another study on morphological and behavioral laterality in crayfish, in which the degree of behavioral laterality had no relation with the degree of morphological asymmetry (Tobo et al., 2012).

Lateralization in the cerebral and neural systems represents internal factors that may affect behavioral lateralization, such as that observed in this study. Cuttlefish possess binocular vision and can assess the exact distance to a target (Messenger, 1968). In our observational tests, each subject faced its prey and watched it with both eyes as it turned. Nevertheless, these animals appeared to utilize one eye preferentially, in correspondence with their laterality. Righty individuals were likely to watch the target shrimp with the right side of their head facing forward in their counterclockwise turn, and lefty cuttlefish watched the prey with their left side of their head facing forward in the clockwise turn. These may be homologous behaviors to the lateralized eye use in octopus (Byrne et al., 2002, 2004), and it is quite possible that cuttlefish also have laterality in eye use. Thus, their turning direction may be dependent on which eye is dominant over the other.

From this study we predict that righty cuttlefish may be more efficient in capturing prey when they turn in a rightward direction than in a leftward direction, and vice versa for lefties. The reason is that lateralized animals have better skill performance and quicker response times than nonlateralized animals due to their cerebral lateralization permitting dual tasks to be performed more efficiently (Rogers, 2000). However, this observation has been made in vertebrate species, and for cuttlefish further study on the relationship

between foraging performance and the dominant side of the body may be needed.

Laterality is generally suggested to have a genetic basis (Vallortigara, 2006, but see Palmer, 2005). Hori et al. (2007) also indicated that structural asymmetry, such as that of skeletal bones, is controlled genetically in fish laterality. It has also been shown that zebra fish and medaka share the same genetic background of laterality (Hata and Hori, 2012; Hata et al., 2012). The abdominal asymmetry of shrimp that relates with their escape direction also has some genetic background (Takeuchi et al., 2008). Although the genetic base of laterality in cuttlefish was not examined in our experiment, we found morphological asymmetry of cuttlebones not only in juveniles, but also in young and adult cuttlefish. Furthermore, juvenile cuttlefish (two weeks after hatching) showed clearly biased attack behavior at first contact with large prey, and their turning direction corresponded with the curving direction of the cuttlebone (personal observation). All of this suggests some genetic basis for laterality in cuttlefish, although crossing experiments are necessary for confirmation.

Cuttlefish show behavioral dimorphism depending on their morphological antisymmetry. Here, we can predict that the prey of cuttlefish, such as shrimp and crabs, likely also have a similar bias in their escape behavior, as seen in atyid shrimps (Takeuchi et al., 2008) and in crayfish (Tobo et al., 2012). In this situation, the dimorphism of cuttlefish may interact with the lateral morphs of prey crustaceans, and the success of predation and/or predator avoidance of each individual may depend on the ambient ratios of right and left morphs of both predator and prey populations. In a field study, Yasugi and Hori (2011) showed that when predators exploit prey of the opposite laterality (predominance of cross-predation), there is an alternating fitness advantage between the two morphs, leading to frequency-dependent selection, which maintains the lateral dimorphism. The potential predators of cuttlefish are large teleost fishes, mammals, and other cuttlefish (Hernández-García, 1995; Hanlon and Messenger, 1998). Although there is a possibility that these predators exhibit lateralized hunting, it is difficult to nominate such predators specialized to cuttlefish. We hypothesize that the laterality of cuttlefish has been maintained by frequency-dependent natural selection through predominance of cross-predation on their prey. A field study is necessary to prove the maintenance mechanism of lateral dimorphism in cuttlefish.

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