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ORIGINAL PAPER

Octopuses (*Octopus bimaculoides*) and cuttlefishes (*Sepia pharaonis*, *S. officinalis*) can conditionally discriminate

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Abstract In complex navigation using landmarks, an animal must discriminate between potential cues and show context (condition) sensitivity. Such conditional discrimination is considered a form of complex learning and has been associated primarily with vertebrates. We tested the hypothesis that octopuses and cuttlefish are capable of conditional discrimination. Subjects were trained in two maze configurations (the conditions) in which they were required to select one of two particular escape routes within each maze (the discrimination). Conditional discrimination could be demonstrated by selecting the correct escape route in each maze. Six of ten mud-flat octopuses (*Octopus bimaculoides*), 6 of 13 pharaoh cuttlefish (*Sepia pharaonis*), and one of four common cuttlefish (*S. officinalis*) demonstrated conditional discrimination by successfully solving both mazes. These experiments demonstrate that cephalopods are capable of conditional discrimination and extend the limits of invertebrate complex learning.

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Introduction

Learning that supports spatial orientation can include recognition of beacons or landmarks, use of directional information provided by large, external cues (e.g. solar or geomagnetic cues), and mechanisms that record internal information (e.g. steps taken, turns made, or energy expended; see Healy 1998; Shettleworth 1998; Golledge 1999). Field data suggest that learning that supports spatial orientation is important to octopuses. Octopuses are central place foragers, basing their activities from a temporary home den. They forage in multiple sites surrounding the den, do not visit the same foraging site on consecutive days, travel distances of up to 40 m (Forsythe and Hanlon 1997), and use return routes different than outbound routes (Mather 1991). Some features of the environment appear to be more salient than others in guiding their movements [e.g. large cliff faces rather than small local cues (Mather 1991)]. These field data all suggest that learning supports spatial orientation in octopuses, and such learning has been confirmed in maze experiments (Schiller 1949; Wells 1964, 1967, 1970; Walker et al. 1970; Mather 1991; Boal et al. 2000). Field data describing cuttlefish behavior is generally limited to reproductive activity (e.g. Corner and Moore 1980; Watanuki et al. 2000; Hall and Hanlon 2002; Naud et al. 2004); nevertheless, cuttlefish also show good evidence for learning in laboratory maze experiments (Karson et al. 2003; Alves et al. 2006, 2007).

Discrimination is the ability to respond selectively to one of several stimuli that are presented either simultaneously or sequentially. Discrimination learning is well established from laboratory experiments using both octopuses (reviewed in Boal 1996) and cuttlefishes (Messenger 1977; Karson 2003; see also Sanders 1975; Mather 1995; Hanlon and Messenger 1996) as subjects. Discriminations between objects can be made using either visual or tactile cues (reviewed in Wells 1978; Hanlon and Messenger 1996). This ability likely supports the learning of both landmarks (Mather 1991) and prey types (reviewed in Hanlon and Messenger 1996; Darmaillacq et al. 2004a, b).

For discrimination abilities to fully support spatial orientation, animals must discriminate between highly specific characteristics of landmarks (this rocky outcrop vs. that rocky outcrop) or use context to differentiate between similar landmarks (the rocky outcrop near the sandy plateau vs. the rocky outcrop by the sea grasses). They must also use the landmark flexibly, since an appropriate response to a landmark typically will differ between outbound and return routes. A control of discrimination through sensitivity to context is referred to as conditional discrimination. More specifically, “conditional stimulus control is produced in situations in which the stimulus–response–reinforcer relations involved in simple discriminations are themselves placed under the control of other (conditional) stimuli” (Mackay 1991). Conditional discrimination (level 5) is considered a form of complex learning (Thomas 1980, 1996) and, among invertebrates, has been previously demonstrated in both honeybees (e.g. Couvillon and Bitterman 1988) and *Aplysia* mollusks (Colwill et al. 1988). (Note that conditional discrimination is not the same as conditional concepts, level 7 in Thomas’ 8-level scale.)

The experiments presented here tested our prediction that cephalopods are also capable of conditional discrimination. Individual octopuses and cuttlefish were trained with two different maze configurations using escape as motivation. For octopuses, large open field mazes contained small, hidden burrows, one of which extended down under the substrate. For cuttlefishes, small round mazes contained two doorways, one of which permitted a return to the large home tank. For all subjects, the escape location in one maze configuration was diametrically opposite to the escape location in the other maze configuration. Conditional discrimination could be demonstrated by a successful mastery of both maze configurations when presented in intermixed trials.

General methods

Subjects

The species used in the present experiments were chosen because of their ready availability. All animals were fed (octopuses every 2 days, cuttlefish daily) a mix of thawed, frozen shrimp, live fish, and live crabs.

Housing

Experiments were conducted from 2002 through 2006. At Millersville University (experiments 1 and 3), all animal housing and experimental tanks were connected to a single 5,500 L marine system of recirculating seawater. Seawater was fabricated from reverse-osmosis, filtered tap water and Instant Ocean brand artificial sea salts (Mentor, Ohio); salinity ranged from 33 to 35 ppt. Water leaving any housing or experimental tank passed through mechanical, chemical, and biological filters, as well as UV sterilization, before returning to any other tank. Water temperature was controlled by a chiller and ranged from 15 to 21°C (a range of no more than 3°C within any one experiment). Natural light from north-facing windows was supplemented with artificial fluorescent lighting during normal workday hours. Octopuses were housed individually in small tanks (20 × 36 × 24 cm deep) that included coarse substrate, a few mollusk shells, and a terra cotta flower pot for a den. Cuttlefish were housed together in a single large tank (183 × 305 × 55 cm deep) with soft sides (Hanley et al. 1999) that included artificial algae, rocks, terra cotta flower pots, and pipe sections.

At the National Resource Center for Cephalopods (NRCC) in Galveston, Texas (experiment 2), cuttlefish were housed in a single, large tank (370 × 610 × 120 cm deep). This tank was part of a closed marine system (65,000 L) that has been described elsewhere (Minton et al. 2001); in short, seawater (natural seawater supplemented with Instant Ocean brand artificial sea salts, Mentor, Ohio) was filtered using mechanical, chemical, and biological filters, as well as UV sterilization. The salinity ranged from 32 to 35 ppt and the temperature ranged from 25 to 28°C.

Mazes and experimental procedures are described below. Each maze was provided with visual, and in some cases tactile, cues located both underwater and above the water’s surface. Cephalopods have excellent visual acuity (Budelmann 1994), and previous research indicates that they perceive cues located out of the water (Boletzky 1972; Boal et al. 2000; Adamo et al. 2006; King and Adamo 2006) as well as under the water. Numbers of trials and inter-trial intervals sometimes varied slightly from day to day, as indicated, because of the constraints of the student-experimenter’s schedules.

Experiment 1

Previous research indicates that octopuses spontaneously explore a novel environment (Boal et al. 2000). Results from extensive preliminary experiments (unpublished data) suggested that both conspicuous local cues and room cues external to the maze are important for orientation. Disorienting the octopuses by gently spinning them in their pots before placing them in the maze improved maze performances, perhaps by increasing motivation to escape; idiothetic orientation did not appear to be important. Stress from handling appeared to compromise learning; thus, the handling of any one octopus was limited to a maximum of twice per day (once into the maze and once out of the maze). Time for exploratory learning (Boal et al. 2000) was provided during the night when this octopus species appears to be most active (Boal 1993) and human activity in the laboratory (and building) generally ceased.

Methods

Subjects

Subjects were *Octopus bimaculoides* (Pickford and MacConnaughey 1949), also known as the two-spotted or mudflat octopus. This species is found from coastal waters of Central California south to the Baja Peninsula (Hochberg and Fields 1980). It is the smaller of two sibling species, the larger of which, *O. bimaculatus* (Verrill 1883), is also known as the two-spotted octopus. The lifespan of *O. bimaculoides* ranges from 12 to 16 months (Forsythe and Hanlon 1988). Octopuses (18–158 g) of unknown age were trapped in the field and shipped to Millersville University for use in experiments.

Experimental apparatus

A maze was constructed from a dark gray, round fiberglass tank (1.46 m diameter × 0.73 m deep). Two burrows (8 cm diameter) were cut into the bottom of the tank 14 cm from the side wall (see Fig. 1). Transparent glass jars (14 cm tall) exactly fit into each burrow. In each maze, one of the two burrows contained an inverted jar, sealing off the entrance ("closed burrow"), and one jar contained a jar oriented right-side-up ("open burrow"), providing an escape from the maze. *Octopus bimaculoides* is a small, shy, predominantly nocturnal species. During trials, it was motivated to escape from the large, brightly-lit arena and enter the small, dark burrow.

Distinctive maze configurations were created using pairs of semi-circular plates onto which various materials were glued (five plates: light smooth, light sand, dark rough, dark pebbles, dark rocky). Other cues included bottom landmarks (artificial algae, large stones, a coil of rope, plastic sand-filled 1-L jugs) and wall cues hung over the side of the tank (light or dark towels, either patterned or solid; arrays of either vertically suspended yellow rulers or round white plastic container lids). Combinations of cues were chosen such that no two octopuses were trained in the same maze configurations, and for any one octopus, no cue appeared in both maze configurations.

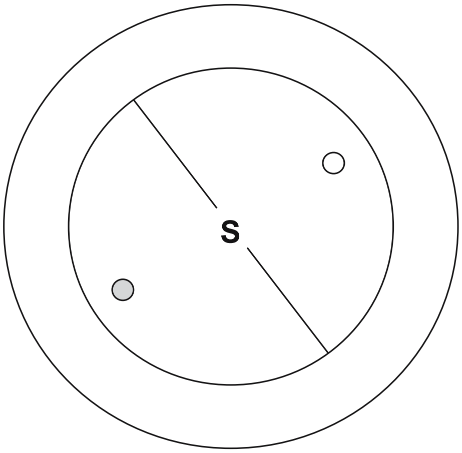

Fig. 1 Schematic of the octopus maze as viewed from above. The maze consisted of a round arena (1.46 m) with two potential burrows (*small circles*). The maze could be configured with a variety cues and landmarks on the walls (*outer ring*) and bottom (*inner circle*). The octopus started in the center of the maze (*S*). For each octopus, only one burrow was open (*open small circle*) while the other was blocked with an inverted glass jar (*shaded small circle*). The maze was large relative to the octopuses; the largest octopus could not reach more than half way from one burrow to the next when its arms were fully outspread. The octopuses were motivated to escape the large, bright arena and enter the small, dark open burrow

The octopus maze was supported within a larger tank (1.83 m diameter × 0.76 m deep). Water flowed into the maze, drained through small holes drilled into the bottom, and passed to the outer tank, which was fitted with a return line to the marine system's filtration units. The depth of the water within the maze (22 cm) was set by an adjustable standpipe in the outer tank.

At the start of trials, a stand (9 cm above the substrate) at the center of the maze allowed the experimenter to invert a terra cotta flower pot, still containing an octopus, into the maze at a standard location. Normally the octopus promptly dropped down into the maze and proceeded to move about the arena. Occasionally, the octopus did not drop down. In this case, a cotton swab, dipped in a 10% bleach solution, was poked into the drain hole of the inverted pot. The octopus promptly dropped down out of its pot and showed no harm from this treatment. At the end of a trial, the right-side-up jar in the open burrow could be removed, while still containing the octopus, providing an easy way to transport the octopus back to its home tank.

Two groups of octopuses were trained. Octopuses in group 1 sometimes traveled near the closed burrow but did not touch it. Because this resulted in potential ambiguity about whether or not the octopus had somehow detected that the burrow was closed, for group 2 white plastic rings that extended 5 cm above the substrate were fixed around each burrow entrance. Burrow entries for group 1 were always preceded by crawling and never by jet-swimming;

5 cm was sufficient to prevent a crawling octopus from seeing into the burrow. To prevent rapid, random jets away from the start location, for group 2 a clear bell was inverted over the start location and was raised remotely using a rope-and-pulley system 30 s after the octopus was placed in the maze. Because the octopus' pot was a distracting safety location, for group 2 the octopus' terra cotta flower pot and the starting stand were removed from the maze two and a half minutes later to encourage the octopus to fully explore the maze.

To record trials, a video camera was mounted on the ceiling directly above the maze and was connected to a computer that was located approximately 5 m away to avoid any distraction to the octopus by the experimenters.

Procedures

One group of octopuses was trained in 2005 ($N = 6$) and a second group was trained in 2006 ($N = 4$). All octopuses received a maximum of five trials in each maze configuration. Octopuses were placed in the maze at 16:00 and removed from the maze at 11:00 the following morning. The first group received one trial per day (ITI = 5 h) while the second group received one trial every other day (ITI = 30 h). For all ten octopuses, the two maze configurations were presented alternately throughout training (a maximum of 10 trials for each octopus). To assess learning, the first 60 min (group 1) or 30 min (group 2) of each trial was recorded for analysis; the time recorded was shortened for group 2 because no useful data emerged from the second 30 min of group 1. To demonstrate conditional discrimination, octopuses needed to solve their last five maze trials correctly (binominal distribution, $P = 0.03$, one-tailed).

Results

Four of the six octopuses in group 1 showed evidence for conditional discrimination (Table 1). One octopus (Zoidberg) traveled directly to the open burrow in trials 4 and 5 in both maze configurations (Fig. 2). Three other octopuses (Ultros, Rocky, Hercules) made no errors on trials 3–4 or 3–5 in either maze; however, some of their approaches were indirect (approached but did not touch the closed burrow; similar to Fig. 2, path A1).

Two of the four octopuses in group 2 showed evidence for conditional discrimination (Table 1). In this group, errors could be scored with full confidence because the octopus needed to reach over the ring and into the burrow to detect whether it was open or closed, behavior that was obvious in recordings. One of these two octopuses (Alaria) was successful on all but one trial. The other octopus (Rukia) was successful on trials 4–5 in maze A and trials 3–5 in maze B (Table 1).

Table 1 In experiment 1, 6 of 10 *Octopus bimaculoides* conditionally discriminated (CD) by learning to escape from two different mazes (A and B)

Octopus	Maze A					Maze B					Time (s)	*N*	*P*	CD?
	1	2	3	4	5	1	2	3	4	5				
Group 1														
Zoidberg	C_i	C_i	C_i	C_d	C_d	X	X	C_i	C_d	C_d	249	6	0.020	Yes
Ultros	C_d	C_d	C_d	C_i	C_d	C_d	C_d	C_i	C_d	C_d	14	10	0.001	Yes
Rocky	X	C_d	C_d	C_i	–	C_i	C_i	C_d	C_d	–	37	6	0.020	Yes
Hercules	C_d	X	C_i	C_i	–	X	C_d	C_i	C_d	–	52	5	0.030	Yes
Zebra	*	*	*	C_i	C_i	*	*	*	C_i	X	94	0	–	No
Winry	X	X	X	C_i	X	X	X	C_d	C_i	C_i	5,000	0	–	No
Group 2														
Alaria	C	C	C	C	C	X	C	C	C	C	461	8	0.004	Yes
Rukia	C	X	X	C	C	X	X	C	C	C	680	5	0.030	Yes
Loki	X	X	X	X	X	X	C	C	C	C	5,000	0	–	No
Beroe	X	X	X	X	X	X	X	C	X	X	5,000	0	–	No

Each maze included two escape burrows, one open (correct) and one closed (incorrect). The mazes were presented alternately for a total of 10 experimental trials. Table entries indicate whether the octopus was correct (C) or incorrect (X) on that trial. For group 1, C_d indicates a direct approach and C_i indicates an indirect approach to the open burrow. A line (–) indicates that no trial was given; an asterisk (*) indicates a recording equipment failure. Median time to escape into the open burrow (Time) is provided for each octopus (5,000 for no escape)

N Number of terminal consecutive correct trials; *P* binomial probability; *CD* whether conditional discrimination was demonstrated

Two octopuses in group 1 (Zebra, Winry) and 2 octopuses in group 2 (Loki, Beroe) failed to show any evidence for conditional discrimination. These octopuses moved around with no apparent direction in at least one maze, and touched a closed burrow before touching the open burrow on many trials (Table 1).

For the six octopuses that showed learning in both mazes, no evidence was found for a reduction in the time taken to reach the open burrow with greater experience (maze A: trial 1 median 69 s, range 8–786 s, trial 5 median 64 s, range 13–438 s, Sign test, $N = 6$, $k = 3$, $P = 0.65$; maze B: trial 1 median 407 s, range 9–1,800 s, trial 5 median 157 s, range 23–704 s, Sign test, $N = 6$, $k = 2$, $P = 0.34$). Time taken to reach the open burrow differed markedly between individuals (Table 1); some individuals moved about rapidly (e.g. Ultros, median 14 s, range 8–57 s) while others moved very slowly with intermittent periods of inactivity (e.g. Alaria, median 461 s, range 251–1,800 s).

Initial headings appeared haphazard. Conspicuous landmarks within the maze were visited frequently, sometimes many times within a single trial, whether the landmarks were near the open burrow or not. During initial trials, octopuses in group 2 often climbed into the ring surrounding the closed burrow before moving on. Errors (touching the closed burrow) were rare in later trials, even among

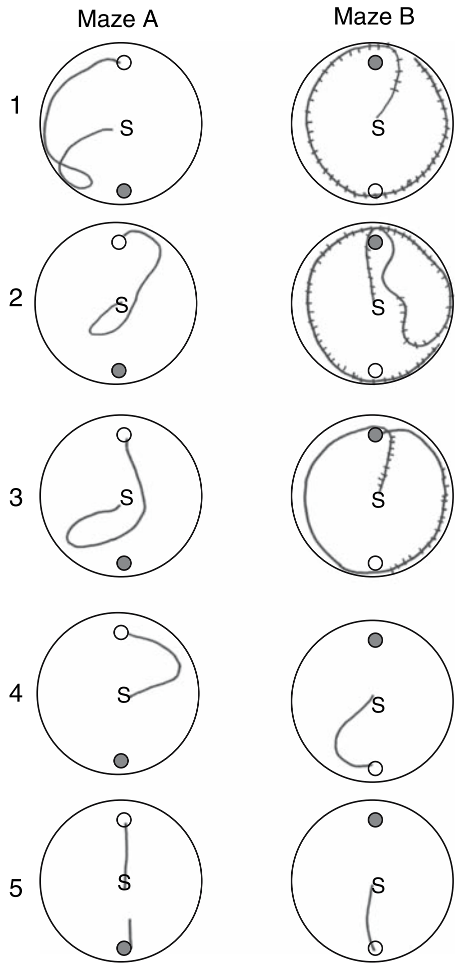

Fig. 2 The path of a single octopus in experiment 1. Each octopus received five trials within each of two mazes (*left column* maze A; *right column* maze B) presented alternately (A1, B1, A2, B2, etc). Each maze contained one open and one closed burrow (*open* and *filled circles*, respectively), various landmarks, and differing substrates on the two halves of the maze (not shown). The octopus was placed in the center of the maze ("*S*") at the start of the trial. The path of one octopus (Zoidberg) is shown, with *smooth lines* indicating crawling and *hatched lines* indicating jet-swimming. The octopus headed directly for the open burrow in trials 4 and 5 of both mazes, indicating conditional discrimination

octopuses that failed to show learning. These octopuses typically jet-swam haphazardly around the maze or settled down near a landmark or at the edge of the maze and did not move. Almost all octopuses in almost all trials were found inside the open burrow the following morning (exception: Loki, first trial in each maze).

Discussion

Six of the ten octopuses showed evidence for conditional discrimination. For three of these octopuses (Zoidberg, Alaria, Rukia), evidence was clear; Zoidberg took a straight path directly to the open burrow in both mazes (Fig. 2) and Alaria and Rukia did not contact the closed burrow, which was out of sight. For three others (Rocky, Ultros, Hercules) evidence was strong but compromised by their approaches (without contact) to the closed burrow (Table 1). It is possible that these three octopuses detected the state of the burrow (open or closed) visually, and used this information to guide their choices. We consider this possibility unlikely. In previous work (Boal et al. 2000; unpublished data) and in this experiment, we found no evidence that octopuses detected an open burrow without touching it. Entries were always preceded by crawling and never by jet-swimming. The octopuses frequently crawled or jet-swam right next to an open burrow and sometimes jet-swam right over an open burrow, but did not touch it, enter it, or backtrack to it, suggesting that proximity alone did not provide useful information. Open burrows were always touched with an arm before entry, and such touches were easily visible on the video recordings. It appears, therefore, that these three octopuses also conditionally discriminated.

Performances of individuals varied widely. Some individuals explored the maze readily and unhurriedly, entering the open burrow only to reemerge a minute or two later for further exploration. Other individuals jet-swam haphazardly around the maze, sometimes crashing into the walls, as if highly stressed. The four octopuses that failed to learn jet-swam frequently, or froze for extended periods by a landmark or by the side of the maze. Those that demonstrated learning mostly crawled around the maze at a moderate pace. Variation in the behavior of individuals is well documented in cephalopods (Mather and Anderson 1993; Sinn et al. 2001; Calvé 2005; Sinn and Moltschaniwskyj 2005; Adamo et al. 2006). Octopus temperament, a new and active topic of research (e.g. Sinn et al. 2001), appears to influence maze performances in this species.

Experiment 2

This experimental design was based on previous research demonstrating maze learning in the common cuttlefish, *Sepia officinalis* (Karson et al. 2003). We are not aware of other learning experiments using *S. pharaonis* as subjects.

Methods

Subjects

Subjects were *Sepia pharaonis* (Ehrenberg 1831), also known as the pharaoh cuttlefish. This species is found throughout the Indo-Pacific, from the Red Sea to Japan and Australia (Gabr et al. 1998). The lifespan of this species is estimated to be 9–18 months (Gabr et al. 1998). Cuttlefish were reared from eggs at the NRCC. Experiments with

S. pharonis (13–17 cm mantle length) were conducted at the NRCC.

Experimental apparatus

The cuttlefish maze was similar to one described in Karson et al. (2003). Briefly, a maze was constructed using a dark-colored plastic barrel (56 cm diameter) with its bottom removed. A new floor for the maze was fabricated from clear Plexiglas™ and installed 60 cm below the top of the maze. During trials, the maze was suspended in the home tank so that the top rim of the maze was 5 cm above the surface of the water and the maze floor was 65 cm above the bottom of the home tank.

A start tube (fully submerged; 16 cm diameter) was placed 17 cm below the top rim of the maze (Fig. 3). Each end of the start tube was fitted with an opaque sliding door. Two holes (20 cm × 15 cm) were cut on opposite sides of the maze, even with and perpendicular to the start tube. Doors, fabricated from clear Plexiglas™ that did not distort polarization (Shashar et al. 1996), were hinged over the holes so that they could swing up (open) or down (closed). Exchangeable fabric panels (36 cm × 36 cm), one spotted and one striped, were placed surrounding the doors using Velcro. Cues (a brick or a piece of artificial algae) were placed in the maze directly opposite the start tube and between the two doorways.

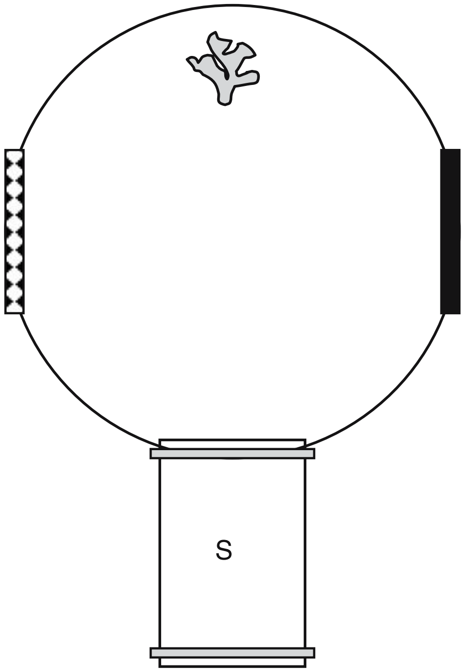

Fig. 3 Schematic of the cuttlefish maze as viewed from above. The maze consisted of a round arena (0.51 m) with a clear bottom. The maze was suspended within the cuttlefish's home tank. Two closeable transparent doors, located opposite one another, were surrounded by exchangeable fabric panels (striped or spotted). On each trial, only one door was open. A piece of PVC pipe, fitted with opaque doors on either end, served as a start tube (*S*). A cue (algae or brick) was placed opposite the start tube, indicating which door was open (spotted or striped). The arena was small relative to the cuttlefish, which nearly filled the start tube by the end of each experiment. The cuttlefish was motivated to exit the small arena through the open door to return to its large home tank and rest on the substrate below

Common cuttlefish are benthic and prefer to rest on the bottom of their tank; the clear floor of the maze was designed to allow the cuttlefish to see out of the maze to the home tank below, but prevent direct access to the home tank. The pharaoh cuttlefish, like common cuttlefish, were expected to be motivated to escape the confined maze and swim down to the bottom of their home tank (Karson et al. 2003).

Procedures

Group-housed pharaoh cuttlefish ($N = 13$; 6 males, 7 females; mantle lengths 13–17 cm) were uniquely identified using banding patterns on their apparent dorsal side.

To test learning, the maze was placed within the cuttlefish's home tank. The location was randomized to ensure that the cuttlefish were not using cues outside of the maze to orient. Cuttlefish were given three to five trials per day with an ITI of at least 1 h. Trials began by ushering a cuttlefish into the start tube with a dip net. After a 60 s (first 11 trials) or 10 s (all subsequent trials) delay, the door in the start tube leading to the maze was opened. The cuttlefish was given 1 min to leave the start tube; if it did not leave, it was herded gently into the maze using the dip net. Each cuttlefish was given 7 min to exit the maze through an open door; if they did not exit the maze within 7 min, they were guided out with the dip net.

The maze was not as aversive to the cuttlefish as expected. To encourage the cuttlefish to leave the maze, a small dip net was placed in the maze directly in front of the start tube 15 s after the cuttlefish entered the arena (starting at the 12th trial). After an additional 15 s, the dip net was shaken up and down at a set rate, using a stop watch. The net was shaken until the cuttlefish finished the trial.

Trials began with preference testing. Both maze doors were open. Preference testing continued until each cuttlefish (a) exited the maze in under 2 min in each of five trials, and (b) used both the left and the right doorways, each at least once. Preference was defined as the fabric panel through which the individual cuttlefish exited the most frequently in these five trials.

In Task 1, cuttlefish were trained against their initial preference (the door with the preferred fabric panel was closed). A randomly chosen half of the cuttlefish was assigned one maze cue (group 1, the brick) and the other half was assigned the other maze cue (group 2, the algae). Thus, for each cuttlefish, a particular cue-plus-fabric-panel combination indicated which doorway was open; direction (left/right) was irrelevant. The criteria set for learning was six out of seven consecutive escapes in less than 1 min. Once the cuttlefish reached the learning criteria, they were trained with the other maze cue-plus-fabric-panel combination (Task 2). Once the cuttlefish reached criterion on the second task, they were tested with trials using the two maze configurations in semi-random order (Task 3; Fellows 1967). To be successful in this experiment, the cuttlefish needed to learn which exit to use, using the cue-plus-fabric-panel combination to discriminate between doorways.

Because of high variability in performances, the median escape time for each cuttlefish in each block of three trials was used in analyses.

Results

All 13 cuttlefish completed preference testing within 23 trials (range15–23). Two cuttlefish preferred the striped pattern and 11 cuttlefish preferred the spotted pattern. Eight cuttlefish had noticeable preferences (4 or 5 out of five trials); these cuttlefish were divided evenly between groups (Task 1—algae, Task 2—brick or Task 1—brick, Task 2—algae). A total of six cuttlefish completed Task 3; all results that follow are for these six cuttlefish.

In Task 1, escape times were erratic but decreased significantly overall (block 1 compared to block 14, the last block completed by all 6 cuttlefish; Sign test, $N = 6$, $k = 0$, $P = 0.03$). Median escape time for block 1 was 81 s (range 45–418 s) while for block 14 it was 47 s (range 21–76 s). The cuttlefish also became more consistent, as shown by a significant decrease in the spread of escape times (maximum time minus minimum time) within each block for each cuttlefish (Sign test, $N = 6$, $k = 0$, $P = 0.03$). The median spread for block 1 was 271 s (range 221–474 s) while for block 14 it was 28 s (range 6–80 s).

Neither time nor consistency improved further between Tasks 1 and 2 or between blocks within Task 2. Task 2 was mastered more rapidly than Task 1 (trials to criterion; Task 1 median 44.5, range 37–52; Task 2 median 11.5, range 6–25; Sign test, $N = 6$, $k = 0$, $P = 0.03$) and percent success was higher in Task 2 than Task 1 (percent of exits in <1 min; Task 1 median 42, range 30–51; Task 2 median 75, range 64–86; Sign test, $N = 6$, $k = 0$, $P = 0.03$).

Performances in Task 3 indicated learning from Tasks 1 and 2 was retained. Neither escape time nor consistency improved further in Task 3. The median number of trials to criterion in Task 3 was 7 (range 7–22) and the median percent success was 75.5% (range 63–87%). All six cuttlefish attained a higher percent success in Task 3 than in Task 1 (Sign test, $N = 6$, $k = 0$, $P = 0.03$); four of the six cuttlefish also had a higher percent success in Task 3 than in Task 2 (Sign test, $N = 6$, $k = 2$, $P = 0.34$). During their criterion trials, five of the six cuttlefish erred on the maze configuration they were trained with in Task 2 (consistent with their initial preference; see Table 2).

Evidence for conditional discrimination was found for all six cuttlefish that reached criterion in Task 3 (6 out of 7 consecutive escapes in <1 min; assuming that such escapes were correct and escapes taking longer were incorrect, the binomial probability of attaining this result by chance alone is 0.05; see Table 2). Four cuttlefish reached this criterion immediately: Samantha and Luna made no errors in their first six trials and McGuyver and Inferno made just one error in their first seven trials. A fifth cuttlefish, WuTang, met the criterion with just one extra trial (8 trials total). The sixth cuttlefish, Isabella, required further learning within Task 3, but reached criterion after 22 trials. Exits in Task 3 were rapid; the average escape time for Luna was just 7 s.

Discussion

All six cuttlefish that completed Task 3 demonstrated conditional discrimination by rapidly exiting from the appropriate doorway in six out of seven consecutive trials when mazes were presented in random order. Four of these cuttlefish reached criterion in the minimum possible number of trials (7 trials). For these four cuttlefish, all errors made were with that individual's second maze configuration (consistent with initial preference). Thus, although criterion was reached more rapidly and with greater percent success in Task 2 (consistent with preference) than in Task 1 (against preference), learning was poorer. Previous experiments showed no significant improvement over a series of reversals when cuttlefish were trained consistent with initial preference (Karson et al. 2003); similar results were found with octopuses (Boal 1996).

A drawback to this experiment was that the criterion for demonstrating learning was defined as exiting the maze in <1 min. Based on previous experiments (Karson et al.

Table 2 In experiment 2, 6 of 13 pharaoh cuttlefish (*S. pharaonis*) reached criterion in the mixed trials of Task 3 (maze A: algae cue; maze B: brick cue)

Cuttlefish	1st maze	# trials	1	2	3	4	5	6	7	Time (s)
Samantha	B	6	B	A	B	B	B	A	(A)	23
WuTang	B	8	A	A	B	A	(A)	B	B	30
Isabella	A	22	B	A	(A)	A	A	B	A	43
Luna	B	6	B	B	B	A	A	A	(A)	7
McGuyver	B	7	B	B	A	B	(B)	B	A	19
Inferno	B	7	B	B	(A)	B	B	B	A	33

Shown are first maze trained, number of trials to criterion (6 of 7 escapes in <1 min), maze presentations on trials to criterion, and average escape time in correct trials. Parentheses indicate errors (escapes that took longer than 1 min)

2003), we thought that 1 min was too short for errors to occur. Although most cuttlefish that touched the closed door did fail to exit the maze in <1 min, occasionally an individual would touch the closed door and then immediately turn and exit the correct doorway. This was not counted as an error or recorded in the data. Does this flaw negate the conclusion that these cuttlefish conditionally discriminated? We think not. Average escape times were quite short for all cuttlefish (Table 2). Luna's average escape time was just 7 s; she could not have touched the closed exit and then swum out of the open exit in that amount of time. She must have conditionally discriminated to achieve this performance.

Although there was no noticeable difference between male and female performances, half way through the experiment the cuttlefish began to mate. The males were clearly becoming more aggressive towards the females. This behavioral change may have affected the stress levels and performances of the female cuttlefish. One female in particular (Wilbur), who was the first cuttlefish to reach criterion for Task 1, started to perform poorly once the mating started. She was the only cuttlefish in group 1 that did not reach criterion in Task 2. She had visible sucker marks and scrapes on her body. All females in the experiment regularly demonstrated interest in remaining within the maze, away from the other cuttlefish. They would approach the open doorway and orient themselves so that they could view their home tank, but not exit promptly. Males also demonstrated this behavior but not as frequently as females.

Experiment 3

In previous research, *Sepia officinalis* had greater difficulty learning a combined-cue discrimination than a discrimination using just a visual or a spatial cue alone (Karson 2003). The purpose of experiment 3 was to examine conditional discrimination using a combination of visual (fabric, object) and spatial direction (left/right) cues in this more commonly studied cuttlefish species.

Methods

Subjects

Subjects were *Sepia officinalis* (Linnaeus 1758), also known as the European common cuttlefish. This species is found in 32–35 ppt coastal waters of the Mediterranean Sea and off the coasts of Europe and North Africa (Boletzky 1983). The lifespan of *S. officinalis* ranges from 1 to 2 years (Boletzky 1983). These cuttlefish were also reared from eggs at the NRCC; however, the cuttlefish (approximately 8–16 cm mantle length during experiments) were shipped to Millersville University at about 4 months of age post-hatching for use in experiments.

Experimental apparatus

The cuttlefish maze was similar to one described in experiment 2. Because the home tank for *S. officinalis* was shallower than the one for *S. pharaonis*, the maze was just 47 cm tall and the bottom of the maze was only 12 cm above the bottom of the home tank.

A preliminary study revealed that the maze was not highly aversive and the cuttlefish often settled on the bottom of the central arena and failed to move for extended periods of time (>20 min). To address this problem, an incandescent lamp (aversive) was hung immediately above the maze arena, the total time allowed for a trial was shortened to 5 min, and only a subset of cuttlefish that left the maze readily in pretraining were selected for experimentation.

Procedures

Group-housed common cuttlefish ($N = 15$; 7 males, 8 females; mantle lengths 13–19 cm) were uniquely identified using markings on their apparent dorsal side.

Trials were conducted as in experiment 2. A single cuttlefish was placed in the start tube, and after a 30 s delay, the door in the start tube leading to the maze was opened. The cuttlefish was given 1 min to leave the start tube; if it did not leave, it was herded gently into the maze using a dip net. If the cuttlefish did not leave the maze within 4 min, a net was tapped on the bottom of the maze near the start chamber to induce its movement. If the cuttlefish remained in the maze after 5 min, the trial was terminated and the cuttlefish was herded gently out of the maze using a dip net. Each cuttlefish received up to four trials per day with an ITI of at least 30 min.

Pre-training trials were administered to be sure cuttlefish used both maze exits before training trials began, and to select individuals that left the maze readily. In pretraining, both doors of the maze were open and no cues were present. Fellows (1967) sequences were used to determine which door was striped and which was spotted. Once a cuttlefish exited the maze in under 2 min, using each doorway at least once, pretraining was considered complete.

Preference testing directly followed, with the maze configured as in pre-training. Once a cuttlefish exited the maze five times in under 2 min, preference testing was considered complete. The doorway (left/right) that was used most was considered the preferred doorway for that cuttlefish. Four cuttlefish that exited the maze promptly in both pre-training and preference testing were selected for further training.

In Task 1, each cuttlefish was trained with its non-preferred door open and its preferred door closed. A random half of the cuttlefish had the brick as their first cue and the striped fabric panel around the open door, and the other half had the artificial algae for their first cue and the spotted fabric panel around the open door. In this experiment, cuttlefish were trained to select an exit based on a combination of direction (left/right), fabric panel (striped/spotted), and cue (brick/algae); all three cues were relevant.

Trials were repeated until the individual cuttlefish had reached the criterion of exiting the maze in less than 1 min in six out of seven consecutive trials. Trials in which the cuttlefish failed to exit the maze at all (within the 5 min allowed) were not counted.

A probe trial was administered to confirm learning, and to ensure that cuttlefish were not using water current, odor, or some other inadvertent cue to exit the maze. In this trial, both doors were open. If cuttlefish chose the trained doorway, they were considered to have successfully mastered Task 1. If not, training continued until they successfully exited the maze twice in under 1 min; the probe was then re-administered. Trials continued until the cuttlefish correctly exited the maze in the probe trial.

In Task 2, the cuttlefish were trained with their preferred doorway open and the previously rewarded doorway (Task 1) closed. The cue not used in Task 1 was used in Task 2. Trials were repeated, as in Task 1, until the criterion (6 out of 7 exits in <1 min) was met and a probe trial was completed successfully.

In Task 3, the cuttlefish received trials in which presentations of the mazes used in Tasks 1 and 2 were intermixed. Fellows (1967) sequences were used to determine task order. Trials continued until the cuttlefish met the learning criteria for both tasks, considered separately.

The criterion used in this experiment did not address the possibility that a cuttlefish could contact the closed door, turn around, and then exit through the open door, all within 1 min. In fact, as in experiment 2, some cuttlefish did do this. In experiment 3, all contacts with doors were recorded; consequently, a posthoc analysis was performed, examining all performances for both kinds of errors: contacts with the closed door and exits in greater than 1 min.

Results

Fourteen cuttlefish completed preference testing in a median of eight trials (range 3–15). Nine cuttlefish turned to the right more often and five cuttlefish turned to the left more often. The four cuttlefish that escaped the maze the most promptly were selected for use in this experiment. These four cuttlefish exited the maze in less than 5 min in a median of 89% of all experimental trials (range 55–95%).

Learning was demonstrated in Tasks 1 and 2. Criterion (6 out of 7 escapes in <1 min, followed by a correct probe trial) was reached in Task 1 in a median of 18 trials (range 14–63) and in Task 2 in a median of 27.5 trials (range 16–32). Assuming that escapes in <1 min were correct and escapes taking longer were incorrect, the binomial probability of meeting criterion followed by a successful probe by chance alone is 0.03. Escape times for individual cuttlefish were consistent between Tasks 1 and 2, although some cuttlefish escaped faster than others (median 90 s, range 27–276 s).

All four cuttlefish completed training in Task 3, receiving a median of 43.5 trials (range 34–66) in both maze configurations.

To assess conditional discrimination, performances in Task 3 were examined using the more stringent, posthoc criterion of six out of seven consecutive, accurate escapes (binomial probability = 0.05). An error was scored any time a cuttlefish touched the incorrect door before exiting the correct door and any time it failed to exit the maze in less than 1 min. Conditional discrimination was demonstrated by one cuttlefish. Its performance on trials 13 to 27 of Task 3 (when it reached this new criterion) was as follows (maze A: algae cue; maze B: brick cue; parentheses indicate errors):

B, B, B, (A), A, B, A, A, A, B, (B), A, A, A, B.

The binomial probability of obtaining this result by chance alone is <0.005, one-tailed. Two cuttlefish reached criterion on their first maze (against preference) but not their second maze, while one cuttlefish reached criterion on its second maze (consistent with preference) but not its first maze. These three cuttlefish did not demonstrate conditional discrimination, therefore.

Discussion

One individual clearly demonstrated conditional discrimination in the mixed trials of Task 3, solving both maze configurations promptly and correctly. This individual's performances were significantly better than chance alone.

General discussion

Evidence for conditional discrimination in three cephalopod species was found using mixed trials of two different maze configurations. In experiment 1, six out of ten mudflat octopuses (*O. bimaculoides*) entered the open escape burrow without contact with the closed burrow in mixed trials; for two of these octopuses, approaches were direct or visual contact with the closed burrow was impossible. In experiment 2, 6 out of 11 pharaoh cuttlefish (*S. pharaonis*) selected the correct escape doorway in mixed trials; one of these cuttlefish did so with extreme rapidity (7 s), eliminating the possibility that it touched the closed door first. In experiment 3, one out of four common cuttlefish (*S. officinalis*) selected the correct escape doorway in 13 out of 15 mixed trials. From these experiments, we conclude that these cephalopods can conditionally discriminate.

At this point, we consider it reasonable to assume that any limitations in the cephalopods' performances were due to inadequate experimental design rather than limited learning ability. Octopuses sometimes failed to move about the maze, jet-swam in what appeared to be random directions, or behaved erratically from one trial to the next. Cuttlefish did not reliably leave the maze without prompting in the form of a hand-moved dip net. In all experiments, sample sizes were small and many of the subjects showed no evidence for learning. In the face of all these problems, we find it impressive that clear evidence for conditional discrimination could be found.

The current experiments reinforce the importance of field data in predicting learning abilities. Context sensitivity to landmarks is reasonable for octopuses that are central-place foragers and return home without retracing their outbound path (Mather 1991). Context sensitivity to contingencies is reasonable for soft-bodied animals that are both predator and prey; recent experiments have shown that cuttlefish suppress body patterns typically used in hunting when a model bird (predator) was flown overhead (Adamo et al. 2006). Further information about cephalopod behavior in the field is sorely needed to inform laboratory experiments addressing cephalopod learning and cognition.

Are cephalopods an example of evolutionary convergence in cognitive abilities between invertebrates and vertebrates? On Thomas' (1980) 8-level scale of comparative learning abilities, conditional discrimination, also referred to as discrimination learning (Gagne 1970) or concurrent discrimination learning (Thomas 1980, 1996), is considered an example of level 5 learning. Learning set formation, "learning to learn" (Harlow 1949), or "win-stay, lose-shift" behavior is also considered an example of level 5 learning (Thomas, unpublished manuscript[1]). Learning set has been previously demonstrated in discrimination problems by octopuses (Young 1962; Mackintosh and Mackintosh 1964) and in maze problems by cuttlefishes (Karson et al. 2003). The current experiments demonstrating conditional discrimination confirm that cephalopods are capable of level 5 learning.

Cephalopods are not the only invertebrates capable of level 5 learning. In the small- but complex-brained honeybee *Apis mellifera*, conditional discrimination has been demonstrated in discrimination problems (Couvillon and Bitterman 1988) and in matching-to-sample problems (e.g. Brown et al. 1998). In the small-brained mollusk *Aplysia californica*, individuals discriminated between two environments, showing enhanced responding to touch in the environment in which touch had been followed by a shock (Colwill et al. 1988). The importance of context to learning is well established (e.g. Balsam and Tomie 1985) and makes good ecological sense. It appears that conditional discrimination is a more widespread learning ability than previously recognized.

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