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Food imprinting and visual generalization in embryos and newly hatched cuttlefish, *Sepia officinalis*

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Juvenile cuttlefish spontaneously prefer shrimp to crabs. This preference can be changed by early visual learning during embryonic and postembryonic life and has been related to a form of food imprinting. Nevertheless, it is unknown whether generalization occurs in conjunction with this early learning process. We found that inexperienced hatchling cuttlefish preferred 'black' to 'white crab' for their first meal, the black ink envelope of cuttlefish eggs did not prevent embryos discriminating between black and white crabs and embryonic or postembryonic visual familiarization to 'white crabs' induced a subsequent visual preference for 'white crabs' over 'black crabs' in the hatchling's first meal. Finally, juvenile cuttlefish previously pre- or postnatally exposed to 'white crabs' preferred 'black crabs' to shrimp, indicating for the first time that prey generalization occurs as early as the embryonic stages in cuttlefish. Such cognitive abilities could confer important adaptive advantages in processing information about prey likely to be available in the egg-laying environment at hatching and in the course of juvenile dispersal.

Perinatal learning is of considerable ecological significance. Young or even embryonic animals may detect and learn features of their environment to shape their early behaviours. This specific kind of early learning, imprinting, was one of the first phenomena tackled by the field of ethology. Since Lorenz (1937) first described imprinting, extensive developmental studies of social preference have been performed in young animals. Filial imprinting is a learning process underlying the formation in young animals of an attachment to, and a preference for, a parent, parent-surrogate or sibling (Bolhuis 1991). Although Lorenz claimed that imprinting had to occur during a critical period and was irreversible, subsequent studies have shown that imprinting preferences can be reversed, and that the critical period is not as strict as previously thought (Bolhuis 1991). Imprinting does not imply a simple stimulus–response association, but rather a representation of the stimulus that can then be used flexibly (Bolhuis 1999). Distinct from filial imprinting, sexual imprinting is involved in the formation of mating preferences that are expressed later in life. In males, initial attachment to the mother is later generalized to choose a sexual partner (Immelman 1972). Generalization is the evaluation of stimuli for similarity on one or more perceptual scales to elicit appropriate responses (Ghirlanda & Enquist 2003). Chicks imprinting to a template generalize (Jaynes 1956, 1958; Cofoid & Honig 1961) within a perceptual scale and respond positively to novel objects sharing features with the imprinted one (e.g. shape, colour). This stimulus generalization in imprinting has consequences not only in the later choice of a sexual partner, but also on filial attachment towards different objects. Imprinting has also been associated with food preferences. Burghardt & Hess (1966) first reported that young snapping turtles, *Chelydra serpentina*, exhibit a strong preference for the first food experienced, and that this preference could be established after only one meal (Burghardt 1967). Although this preference was initially characterized as food imprinting, ingesting the first meal may be perceived as a reward, making food preference a result of an associative-learning process distinct from imprinting (Punzo 2002). Since generalization occurs in the context of filial and sexual imprinting, it could be that this process also occurs in the context of food imprinting to aid in the sorting of potential prey.

In young domestic chicks, *Gallus gallus domesticus*, the formation of filial preferences involves at least two processes: a learning process through which chicks come to recognize the features of certain stimuli to which they are exposed and a predisposition to

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approach stimuli resembling conspecifics (Johnson et al. 1992). More than a predisposition, chicks also show a natural preference for brightness that is so stable that it cannot be eliminated by incubating, hatching and rearing in the dark (Zolman & Lattin 1972). Such natural or so-called innate preferences have also been demonstrated in pollinators: honeybee, *Apis mellifera*, workers have a natural colour preference that can be extinguished by training (Giurfa et al. 1995). This combination of innate preference and learning may demonstrate ubiquitous cognitive processes that control foraging behaviour in pollinators and possibly all insects.

The cuttlefish, *Sepia officinalis*, is a sophisticated marine invertebrate that is able to hunt very early in life. Early juveniles are active predators feeding on different types of prey, including fishes and crustaceans, crabs and shrimp (von Boletzky 1983; Dickel et al. 1997). Darmaillacq et al. (2006a) demonstrated that an early visual exposure to prey modifies the food preference of newly hatched cuttlefish for its first meal, several days after hatching. In a two-way choice test (crabs versus shrimp), a 'naïve' cuttlefish preferred shrimp for its first meal, whereas a cuttlefish that had previously seen crabs preferred crabs. This early familiarization has been referred to as food imprinting (Darmaillacq et al. 2006b) since the behaviour includes all characteristics of imprinting. Namely, this familiarization to crab must occur during a sensitive period that ends a few hours after hatching, the learned preference for crabs is persistent, the preference overrides direct food experience with shrimp, and there is no reinforcement during learning (i.e. during the familiarization crabs are not captured). This preference for crabs can also be induced via visual familiarization to crabs during the last stages of embryonic development (Darmaillacq et al. 2008). Any flexibility, however, in learned preferences arising from food imprinting is still uncharacterized. Can generalization occur around these preferences? There is evidence that adult *Octopus* generalize stimuli during visual conditioning: Muntz (1962, 1965) demonstrated that *Octopus* generalize between two squares on the basis of their size. Whether cuttlefish also generalize among stimuli during imprinting is unknown. Cuttlefish are able to discriminate between shrimp and crabs (above), but in those experiments the size of the crabs, *Carcinus maenas*, was controlled (carapace width 2–4 mm). Other characteristics of the prey phenotype may be relevant in imprinting: the human brain clearly classes different groups of young *C. maenas* on the basis of the brightness of their carapace. The first aim of our study was to determine whether hatchlings show a spontaneous preference for a specific crab group and, if so, whether this spontaneous preference can be changed by prior familiarization with a nonpreferred group (experiment 1). The second aim was to determine whether cuttlefish generalize a learned crab group with an unfamiliar one (experiment 2). Experiment 2 was conducted with juvenile cuttlefish, with familiarization to crabs occurring both prehatching and posthatching to determine whether there is an effect of hatching on visual generalization.

METHODS

Cuttlefish lack paralarval or larval stages (von Boletzky 2003), and juveniles have the same morphological traits as adults. Basic behaviours are fully developed upon hatching. As the hatchlings do not receive any parental care (Richard 1971), they must quickly learn to identify prey, shelters and predators. For the present study, *S. officinalis* eggs were obtained from egg clusters that were originally collected from cuttlefish traps, checked daily at low tide, in the vicinity of Luc-sur-Mer, Normandy, France. When performed by competent technicians, this trapping method is by far the least stressful method of collection. Eggs were kept in a 1500-litre tank at the Centre de Recherches en Environnement Côtier (CREC), Luc-sur-Mer, France. Eggs initially laid in clusters were separated to ensure optimal developmental conditions and were placed in strainers floating in laboratory tanks. The tanks were supplied with flowing, oxygenated natural sea water maintained at 19 ± 1 °C (mean $\pm$ SD) under ambient daylight conditions. All experiments comply fully with current laws in France.

Crabs and shrimp were collected daily in the field at low tides from locations where female cuttlefish naturally lay their eggs. Small shrimp of suitable size (*Crangon crangon*, 5–10 mm length) were captured with specially designed soft nets in small pools and kept in a circular tank, 30 cm in diameter and 10 cm deep, in the laboratory. Small *C. maenas* were taken by hand from under rocks and kept in the laboratory in half-submerged boxes with empty shells, pebbles and shelters (15×20 cm) to provide both marine and terrestrial resting spots. All animals were collected by authorized scientific technicians and researchers. After experiments, the cuttlefish were released into the sea at low tide in small sandy pools in which prey were abundant. The crabs and the shrimp used as prey were used after the experiments to feed other cuttlefish reared in the laboratory.

Todd et al. (2005) distinguished eight different phenotypes of *C. maenas*, on the basis of the brightness of the patterns on their carapace. Two of these were used in the present experiments: small crabs with a dull brown carapace ('black crabs') and small crabs with white spots covering at least half of the carapace surface (Fig. 1, 'white crabs'). These crabs were then sorted by size (3–5 mm carapace width). As seen in Todd et al. (2005) and from our own observations of specimens under a binocular microscope, the crabs were identical in behaviour and morphology except for the brightness of their carapace.

Familiarization Protocols

For prehatching familiarization experiments, a square box made of black PVC (50×50 cm and 12 cm high) was divided into 25 cases ($10 \times 10 \times 10$ cm) that were used as individual compartments to isolate eggs. In each case, the floor was pierced in the middle (2 cm in diameter); this hole was surrounded by a glass box in which one

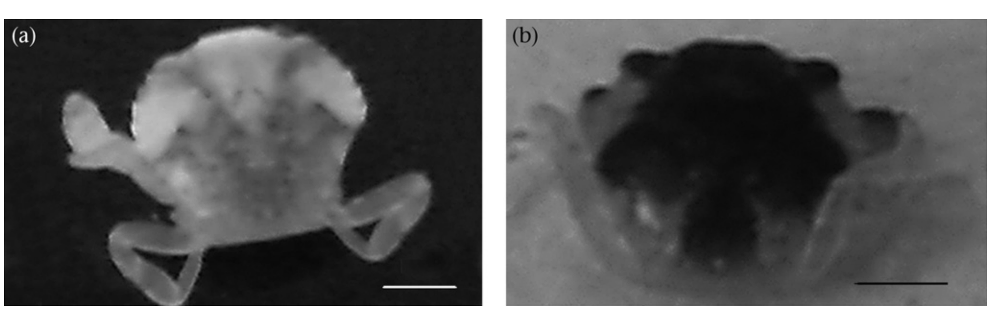

Figure 1. Photographs of the (a) 'white' and (b) 'black' phenotypes of *Carcinus maenas*. Both crabs are 3 mm wide. (Scale: line = 1 mm).