

Ovipositional Host Plant Preference of *Sepia officinalis*

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The present study was undertaken to verify ancient data in the literature on ovipositional preference of the cuttlefish *Sepia officinalis* for the terrestrial plant *Pistacia lentiscus* (mastic tree). – Adult specimens of *Sepia* were placed in basins together with aerial parts not only of *P. lentiscus*, but also of *Artemisia arborescens*, *Centaurea alba* var. *deusta*, *Quercus ilex* and *Laurus nobilis*. The egg masses deposited on the plants were quantified on a daily basis during the 16 day observation period. By far the largest amounts of eggs were found attached to the stems and petioles of *P. lentiscus* (51.8%), but *Q. ilex* and *A. arborescens* also scored high as ovipositional host plants (23.1 and 20.2% respectively). *L. nobilis* and *C. alba* var. *deusta* ranked very low (3.6 and 1.3% respectively). Considering the extremely divergent shape of the three preferred oviposition substrates, which excludes affinity for a uniform type of plant, the possibility of chemical attraction cannot be ruled out.

Introduction

It has been known for many years that the cuttlefish *Sepia officinalis* attaches its eggs to various supports sticking out of the sea floor, in particular to branches of some corals e.g. *Gorgonia*, *Antipathes*, *Isidella* and to tubular worms such as *Spirographis*. In one rare case eggs were found fastened to the tentacles of a dead congener [1]. Fishermen in the Bay of Naples, Italy, have exploited the fact that twigs of various terrestrial plants when immersed in the sea, attract adults of the cuttlefish. In particular branches of *Pistacia lentiscus* (mastic tree) belonging to the family Anacardiaceae, exert a powerful attraction [2, 3]. Apparently this artisanal fishing technique has also been applied by fishermen in other regions of the Mediterranean [4]. To attract mature animals, bow traps garnished with *Pistacia lentiscus* are sunk in shallow littoral waters [4, 5].

The reason why the cuttlefish is attracted by *P. lentiscus* as a host plant for oviposition remains unelucidated. Besides visual perception of the plant by *Sepia*, chemical stimuli emitted by the plant could also be operational in this case of ovipositional preference.

The primary objective of this study was to verify the alleged ovipositional preference of *Sepia officinalis* for *Pistacia lentiscus*. To that end mature

cuttlefish were placed in the presence not only of *P. lentiscus*, but also of a series of other, taxonomically unrelated plants.

Materials and Methods

The experiments were carried out at the Stazione Zoologica "Antonio Dohrn", Naples, Italy, between the end of May and the middle of June 1989.

Sexually mature specimens of *Sepia officinalis* taken by trawls, were obtained from the local fishermen. They were placed in a circular basin with a circumference close to 640 cm, filled with natural seawater to a level of about 45 cm. The animals were fed small crabs every other day. The basin was connected to a flow-through system of natural seawater. Average water temperature was 20 °C. During the 16 day long observation period the cuttlefish population varied between 8 and 12 individuals, with a ratio of 1 ♂/3 ♀♀. Branches of plants collected along the roadside between Mergellina (Naples) and Posillipo were briefly washed before being suspended in the basin from a rod and weighed to keep them in perpendicular position. They were replaced daily by fresh specimens. To avoid position bias in the basin, a randomization scheme was adopted, in which the plant species were each assigned an integer. The position of five plant species on the overhead rod was selected by means of a table of random numbers [6]. The following species were tested as potential hosts for ovipositing cuttlefish (Fig. 1): *Pistacia lentiscus* L.

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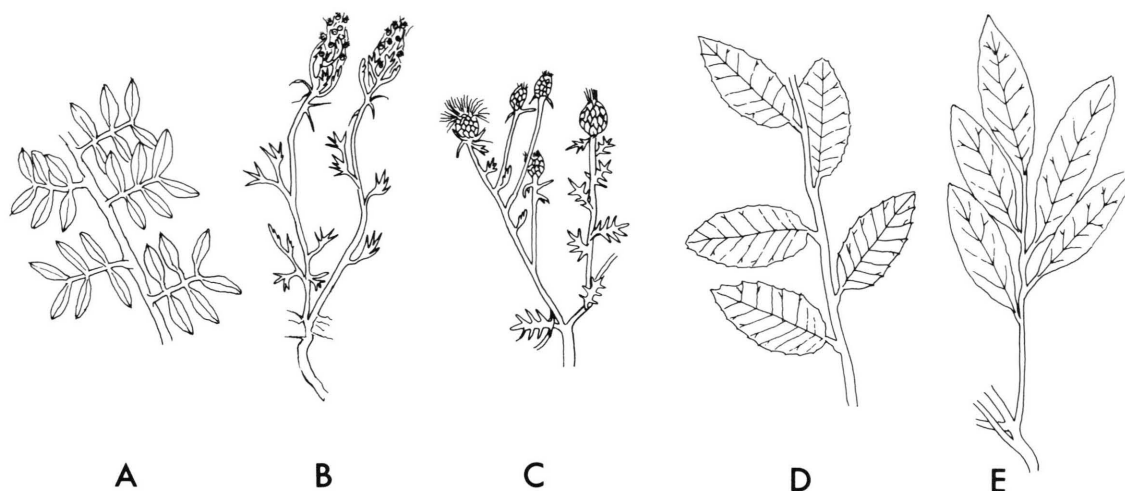


Fig. 1. Diagrams showing the aerial parts of (A) *Pistacia lentiscus*, (B) *Artemisia arborescens*, (C) *Centaurea alba* var. *deusta*, (D) *Quercus ilex*, (E) *Laurus nobilis*.

(Anacardiaceae), *Artemisia arborescens* L. (Compositae), *Centaurea alba* L. var. *deusta* (Ten.) (Compositae), *Quercus ilex* L. (Fagaceae) and *Laurus nobilis* L. (Lauraceae). Only *A. arborescens* and *C. alba* var. *deusta* had flowers. General characteristics of these plants are summarized in Table I.

Bunches of eggs were removed from the branches on a daily basis, and weighed. For statistical analysis Friedman's test was applied, followed by a comparison of paired scores, using Wilcoxon's signed rank test.

Results

Eggs were deposited in the characteristic form of black "sea grapes", whereby each one was fixed by basal processes of the chorion to the stems of twigs, or the petioles. The average size of freshly laid eggs with the envelopes was 8×6 mm. Oviposition took generally place in the late afternoon. Eggs were mostly found attached to the main vertical stem of the plants, but in several instances the petioles were also carrying eggs. The rapid changes that *A. arborescens* undergoes after immersion in seawater (Table I) did not divert the females from ovipositing on this species: eggs were attached not only to the main stem, but also between the lacinate leaves which form flaccid amorphous masses upon contact with seawater. Females were often

seen in the close proximity of the immersed plants (in particular of *P. lentiscus*), stretching their arms in the direction of the stems, also when no oviposition took place.

From an examination of the total amount of eggs attached to the submerged branches (Table II) it appears that more than half the amount had been deposited on *P. lentiscus*. About one fifth of the total egg mass was recovered from *A. arborescens* and *Q. ilex* respectively, and much less from *C. alba* var. *deusta* and *L. nobilis*. The daily distribution of eggs over the five species of host plants (Fig. 2) indicates that *P. lentiscus* was a host without interruption during the entire observation period, i.e. 16/16 times. *A. arborescens* and *Q. ilex* were less regular host plants, 13/16 and 12/16 times respectively. Far behind as regularity is concerned were *C. alba* var. *deusta* and *L. nobilis*, with 2/16, 2/16 times each. There were considerable daily variations in the amounts of eggs deposited.

A non-parametric analysis (Friedman's test) of the daily egg masses permits the conclusion that the differences in weight are not accidental ($P < 0.001$). When pairs of plants are compared with respect to the attached egg masses (Wilcoxon's signed rank test) three groups of plants can be distinguished, each of which shows no significant difference ($P > 0.05$): 1. *P. lentiscus* + *Q. ilex*, 2. *Q. ilex* + *A. arborescens*, and 3. *L. nobilis*

Table I. Characteristics of potential host plants for ovipositing cuttlefish.

Species	Average diameter of stem [cm]	Color and aspect of stem	Aspect of foliage	Fragrance	Resistance to seawater
<i>P. lentiscus</i>	0.5	dark, rounded	leaves paripinnate, leaflets $\pm 1.8 \times 0.6$ cm, lanceolate to obovate-lanceolate, mucronate, coriaceous	strongly aromatic	very good
<i>A. arborescens</i>	0.3	ligneous, white, tomentose	leaves laciniate, the upper mostly undivided, $\pm 2 \times 0.2$ cm	moderately aromatic	soon after immersion leaves loose turgor, stick together
<i>C. alba</i> var. <i>deusta</i>	0.3	greenish, angular, paniculately branched, with long branches	leaves soft, tomentose, undivided, lower 2-pinnatifid, 1–2 cm long, with oblong-lanceolate segments	no smell	becomes slimy after 2–3 days
<i>Q. ilex</i>	0.5	dark, rounded	leaves oval, sinuate $\pm 4.5 \times 2$ cm, dark-green above, grey-tomentose below, slightly toothed	no smell	good
<i>L. nobilis</i>	0.3	greenish, rounded	leaves coriaceous, $\pm 6.5 \times 2$ cm, oblong, lanceolate, glabrous	moderately aromatic	good

Table II. Weight of eggs deposited on host plants during the 16 day observation period.

Name of host plant	Egg weight
<i>Pistacia lentiscus</i>	244 g (51.8%)
<i>Quercus ilex</i>	109 g (23.1%)
<i>Artemisia arborescens</i>	95 g (20.2%)
<i>Laurus nobilis</i>	17 g (3.6%)
<i>Centaurea alba</i> var. <i>deusta</i>	6 g (1.3%)
Total	471 g (100%)

Differences in weight are not accidental, $P < 0.001$ (Friedman's test). Values connected with the same vertical line are not significantly different, $P > 0.05$ (Wilcoxon's signed rank test).

+ *C. alba* var. *deusta*. The order of ovipositional preference appears as follows: *P. lentiscus* > *Q. ilex* > *A. arborescens* > *L. nobilis* > *C. alba* var. *deusta*.

Discussion

The experiments were intentionally performed with plants of very different morphology and taxonomic position (Fig. 1). This holds in particular

for *P. lentiscus*, *A. arborescens* and *Q. ilex*, the three preferred host plants. Only the first and the second are considered aromatic plants, *Q. ilex* is not. Of course one should not assume tacitly that a plant which yields a fragrant smell recognized as such by man, is also aromatic for cuttlefish. Experiments with *Octopus* have shown that the animal readily learns to discriminate between chemical stimuli, undetectable to us [7]. Why the Neapolitan fishermen adopted *P. lentiscus* as an attractant for *S. officinalis* [2] is not known. Perhaps the surprising resistance of *P. lentiscus* to seawater, as well as unsuccessful attempts with other plants, have determined the final selection. Besides, in the circummediterranean countries the mastic tree is renowned since Antiquity for its resinous odor.

If host plant selection is mediated by an attractant (whether or not aromatic), it is logical to assume that the chemical stimulus is perceived by receptor organs. The so-called olfactory pits in *Sepia* consist of small ciliated cavities situated posterior to the eyes and connected to comparatively large nerves. Their function is supposed to be that of the osphradium [8]. This chemoreceptor organ which

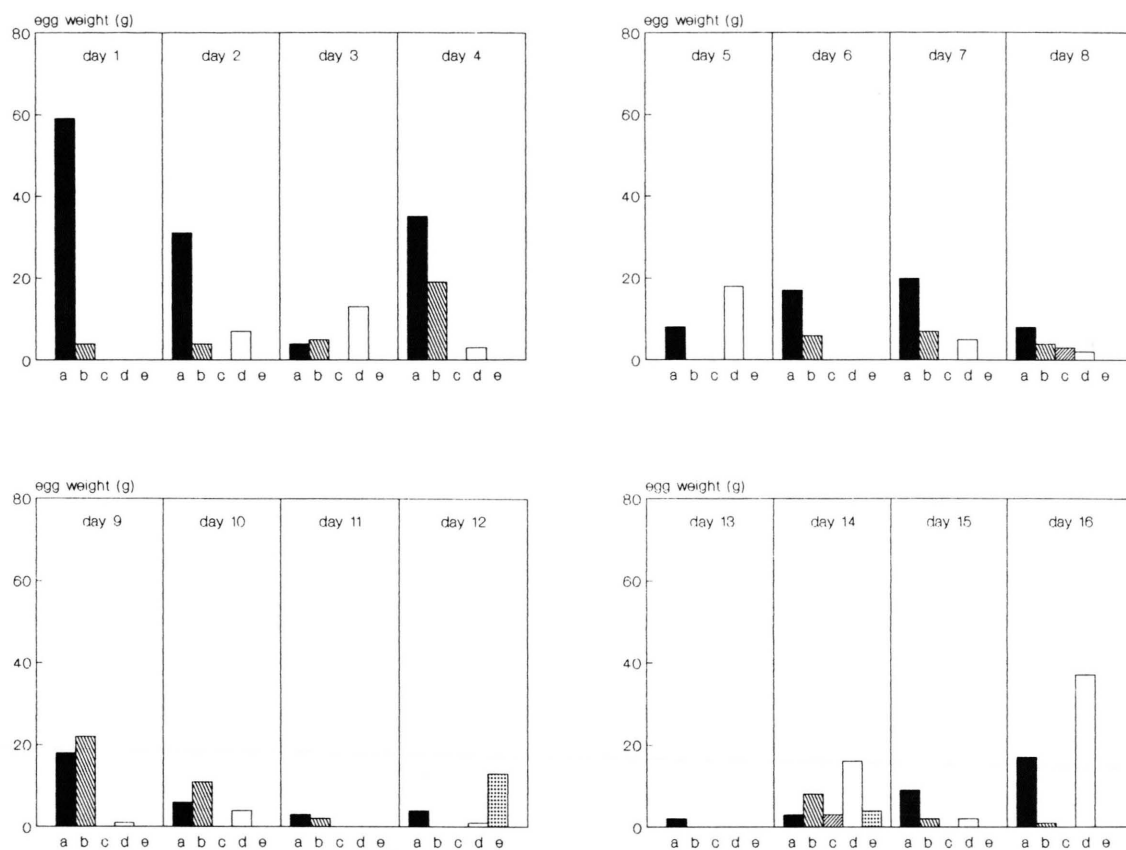


Fig. 2. Histograms of the ovipositional preferences of *Sepia officinalis*. a. *Pistacia lentiscus*, b. *Artemisia arborescens*, c. *Centaurea alba* var. *deusta*, d. *Quercus ilex*, e. *Laurus nobilis*.

is highly developed in prosobranch carnivores is capable of locating carrion, animal juices or prey from a considerable distance, *e.g.* 2 m [9]. But the assumption that cephalopodan olfactory pits have indeed a function in olfaction is not generally accepted. Messenger [10] pipetted sardine, prawn and crab juice onto the olfactory organ of *Sepia* and noticed that this organ was no more sensitive to chemical stimuli than other areas of the animal. It was concluded that if the olfactory pit is a chemoreceptor it probably responds only to specific substances. On the other hand the ultrastructure of the organ (at least in *Octopus*) pleads for a role in chemoreception [11]. Already in the last century fishermen in Greece utilized crushed parts of *Cyclamen (graecum?)* to chase *Octopus* out of their holes [12], which indicates receptivity for chemical stimuli of plant origin. As the epithelium of the

arms of *Sepia*, and in particular of the suckers is abundantly supplied with chemoreceptor cells, the latter could very well be sensitive to and discriminate chemical substances emitted by the submerged plants. Although the animals could hide comfortably in the darker areas of the basin, they preferred to be in close vicinity of the branches, which did not offer much of a hide-out.

Visual stimuli cannot be ruled out as the primary cue in the searching for an oviposition substrate. It has been reported that cuttlefish recognize objects they have encountered before [13]. *Sepia* attaches the eggs to any elongated, thin non-living object such as glass or plastic rods, insulated electric wires, pieces of cord, and celluloid tubing, which obviously do not release chemical stimulants. That *Sepia* would visually recognize its eggs [14] cannot explain the ovipositional preference

for certain host plants, as the female which starts ovipositing has already selected the host plant before the first egg emerges from the funnel.

The experiments described in the present study were performed with plant species which under normal circumstances are not found in the habitat of *Sepia*. The females were therefore not influenced in their ovipositional behavior by previous contacts with these plants, or by habituation. Eggs were attached to three plant species, very different from the point of view of shape, odor and resistance to salt water. In the absence of visual attraction by a given uniform pattern, the ovipositional preference of *Sepia officinalis* could very well be guided by chemostimulants released from the immersed plants. As the strongest attraction was exerted by *P. lentiscus*, current studies are concentrating on this species.

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