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Hatching glands in cephalopods – A comparative study

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ABSTRACT

Hatching of embryos from their eggs involves either mechanical and/or chemical support. In particular enzymes are widely used in the animal kingdom to weaken the egg layers and facilitate the embryo's escape. Although numerous morphological and biochemical studies exist on the hatching glands of invertebrates (such as sea urchins, ascidians, insects) and vertebrates (teleosts, amphibians, and mammals), little is known about the morphology of the hatching glands (Hoyle organs) in cephalopod hatchlings.

In this study, the internal gland structure and the external appearance of the Hoyle organ are compared among several cephalopod species (*Idiosepius pygmaeus*; *Euprymna scolopes*; *Sepia officinalis*; *Loligo gahi*; *Sepioteuthis lessoniana*; *Architeuthis* sp.; *Octopus vulgaris*; *Tremoctopus gracilis*; *Argonauta hians*). In almost all cases the glandular system is restricted to the posterior part of the dorsal mantle surface. Only *Octopus* and *Argonauta* lack a specific glandular structure in this body region and the animals apparently use other mechanisms to penetrate the egg layers.

In all decapod species (*Idiosepius*; *Euprymna*; *Sepia*; *Loligo*; *Sepioteuthis*; *Architeuthis*) as well as in *Tremoctopus* only one specific cell type is present in the Hoyle organ, which synthesizes granular material. The secretory droplets are more or less uniform in electron density in *Idiosepius*, *Euprymna* and *Tremoctopus* but exhibit translucent inclusions in the other decapods. The time of gland development, first synthesis of secretory products and later degeneration after hatching vary between the species.

The present study contributes to our knowledge of glandular systems in cephalopods and allows comparison with hatching structures in other invertebrates and vertebrates.

1. Introduction

Embryos that develop within a protective egg shell need a strategy to hatch from the enclosure. Physical and/or chemical mechanisms are required to penetrate and hatch through the rough protective coats if maternal help is absent. In vertebrates as well as invertebrates the most common actions include muscular movement, a blow of the tail, and increased osmotic pressure of the perivitelline fluid, usually combined with the secretion of enzymes, which attack the chorion membrane.

In cephalopods, mechanisms effective in the process of hatching are muscular contractions (von Orelli, 1959), action of stiff bunches of rodlets (Kölliker's tufts) (von Querner, 1927; Fioroni, 1962b; von Boletzky, 1966; Brocco et al., 1974) and enzymes released from the hatching gland (termed the Hoyle organ) (von Orelli, 1959).

The Hoyle organ (HO) has been demonstrated in several cephalopod species (*e.g. Loligo* sp., *Sepia* sp., *Todarodes* sp., *Sepiella* sp.) (Hibbard, 1937; Arnold, 1965; Matsuno and Ouji, 1988; Shigeno et al., 2001a) during embryonic development. It is present exclusively on the posterior part of the dorsal mantle surface and solely in the late embryonic phase (*e.g. Sepia* sp. from stage 22 to 23, *Loligo* sp. from stage 28, *Octopus* sp. from stage 13 (von Orelli, 1959; Fioroni, 1962a; Arnold and Singley, 1989). In *Sepia* and *Loligo* the HO has an anchor-like shape; in Octopods it forms a slender and small band on the posterior mantle pole.

In cross-section the hatching gland of *Sepia* appears drop-shaped, and its individual secretory cells are filled with granular material (named "ferment" by von Orelli (1959)). Two further cell types are present in the HO; they were drawn but not discussed by von Orelli (1959).

The cells of the HO in *Loligo* are similar to those in *Sepia*, but more compressed. Based on studies by Arnold and Singley (1989) three cell types can be distinguished and are named alpha, delta and mucous cells. The alpha cells, filled with fine filamentous material and many small vesicles, have a dome-shaped apex covered with densely packed microvilli. The delta cells are filled with granules.

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The mucous cells are not restricted to the HO but distributed over the entire surface of the embryo. The authors indicate that the alpha cells are adhesive and attach the HO to the inside of the chorion to localize the effect of the digestive enzymes secreted by the delta cells.

The digestive cells (Fz) in the HO of *Octopus* are elongate and interconnected with glandular (Dz) and epidermal cells. No information is available on the morphology and secretory composition of the glandular cells (von Orelli, 1959).

Following hatching, the production of secretory material ceases and the HO degrades within hours or a few days in all species (von Orelli, 1959).

Although the origin and function of the hatching gland in cephalopods have been known for more than 70 years, knowledge about its morphology is fragmentary. In the present study ultrastructural analyses and X-ray microtomography imaging is used to characterize and compare the HO of nine cephalopod species. For a better comparison and correlation of the morphological data, we provide some additional details about the "egg morphology" during development and at the time of hatching and behavioral observations during the hatching process.

The results help to understand the morphology of glandular cells and allow a more detailed comparison among cephalopods as well as with other invertebrates and vertebrates which develop such structures.

2. Materials and methods

Hatchlings and late embryonic stages of six Decapodiformes (*Idiosepius pygmaeus*; *Euprymna scolopes*; *Sepia officinalis*; *Loligo gahi*; *Sepioteuthis lessoniana*; *Architeuthis* sp.) and three Octopodiformes (*Octopus vulgaris*; *Tremoctopus gracilis*; *Argonauta hians*) were collected with the help of our colleagues worldwide (see acknowledgements). For the morphological comparison always the latest developmental stage of the Hoyle organ (determined by the first sign of secretory activity) was used, except for *Sepioteuthis lessoniana*, *Architeuthis* sp. and *Tremoctopus gracilis*. Specimens of these three species were given as a gift (see acknowledgement) and the specific developmental stages were not verified.

2.1. Ultrastructure

For ultrastructural analyses the samples were fixed in 2.5% glutaraldehyde with a sodium-cacodylate buffer (pH 7.4, plus 10% sucrose) for 6 h at 25 °C. The three museum samples (*Sepioteuthis*, *Architeuthis* and *Tremoctopus*) were stored earlier in 50% isopropanol and also post-fixed for the present study in 2.5% glutaraldehyde with sodium-cacodylate buffer. Subsequently all samples were immersed for 1.5 h in 1% osmium tetroxide with 0.1 M buffer solution and dehydrated in a graded series of ethanol.

For transmission electron microscopy (TEM) the samples were embedded in epon; ultrathin sections (50–70 nm) were mounted on formvar coated copper slot grids, stained with uranyle acetate and lead citrate (Reynolds, 1963), and examined in a Zeiss EM 902 and Zeiss Libra 120.

For scanning electron microscopy (SEM) the samples were washed several times in 100% acetone, dried in a critical point dryer Leica CPD 300, mounted on stubs, coated with gold in a Polaron 5800 sputter coater, and viewed in the SEM Philips XL 20.

2.2. X-ray microtomography (μCT)

For μCT the glutaraldehyde-fixed samples were washed in aqua dest. and dehydrated in a graded series of ethanol. Subsequently, the samples were contrasted for 36 h in 1% (w/v) phosphotungstic acid (PTA) in EtOH and transferred to water for scanning. The

Table 1
Overview of the relevant morphological features relating to the hatching gland of the observed species. In *Octopus vulgaris* and *Argonauta hians* we could not detect any specific glandular structures. Symbols: n.o., not observed; #, not existent; *, The quoted data correlate with the earliest stages available. An earlier occurrence of hatching gland cells is to be assumed. **, Since the cultivated animals were not observed 24 h per day, the hatching time is not detected exactly and therefore the stated degradation time may differ slightly.

	Decapodiformes						Octopodiformes		
	Idiosepius pygmaeus	*Euprymna scolopes*	*Sepia officinalis*	*Loligo gahi*	*Sepioteuthis lessoniana*	*Architeuthis* spec.	*Octopus vulgaris*	*Tremoctopus gracilis*	*Argonauta hians*
Entire shape	Anchor shaped	Anchor shaped	Anchor shaped	Anchor shaped	Anchor shaped	Anchor shaped	#	Single band posteriorly	#
Shape (cross section)	Triangular	Undetermined	Oval to circular	Triangular	Semicircular to lenticular	Triangular	#	Scattered solitary cells	#
Surface appearance	Slightly elevated	Elevated	Distinctly elevated	Slightly elevated	Distinctly elevated	Slightly elevated	#	No elevation indicated	#
Lateral boundary of the gland	Cells with long microvilli	No specific boundary cells	Ciliated cells	Ciliated epithelial cells	Ciliated epithelial cells	Ciliated epithelial cells	#	#	#
Number of secretory cell types	1	1	1	1	1	1	#	1	#
Surface contact of the gland cells	Covered by epithelial cells	Covered by epithelial cells	Direct contact; microvilli border	Direct contact; microvilli border	Covered by epithelial cells	Covered by epithelial cells	#	Covered by epithelial cells	#
Granules appearance	2 μm; globular	2–3 μm; globular	2 μm; globular	2 μm; globular	1.5 μm; globular	2 μm; globular	#	1 μm; globular	#
Hollow areas within granules	#	#	Internal	Peripheral	Peripheral	Peripheral	#	#	#
Other cells within the HO	#	Unspecific epith. cell	#	#	#	#	#	#	#
Stage of first appearance	25*	25*	23	25*	n.o.	n.o.	#.	n.o.	#
Degradation time**	~1 day	>2 days	<2 days	~1 day	n.o.	n.o.	#	n.o.	#