

COMPARISON OF ADHESIVE ORGAN OF *IDIOSEPIUS* SP. AND *EUPRYMNA SCOLOPES*

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Abstract: Histological, histochemical and ultrastructural methods were applied to elucidate the nature of the secretion in the epithelial cells of three *Idiosepius* species (*I. biserialis*, *I. paradoxus* and *I. pygmaeus*). Previous analysis of the adhesive organ of *Euprymna scolopes* by Singley (1982) reveals that adhesion and de-adhesion is caused by a duo-gland adhesive system. The epithelium of *Idiosepius* was studied to elucidate its morphology and the nature of its secretion. The current results show that the adhesive organ of *Idiosepius* consists of three different glandular cells and two non-secretory cell types. Histochemical results indicate that each glandular cell type contains sugar, associated with proteins. The nature of the secretory products suggests that all cell types are responsible for adhesion. Acid proteins were not found in the adhesive organ of *Idiosepius*. A duo-gland adhesive system as in *Euprymna scolopes* can therefore be excluded for *Idiosepius*. *Idiosepius* presumably uses the “Stefan-type” of adhesion. This form of adhesion involves the presence of two flat surfaces with a thin film of liquid in between. The different adhesive substances and mechanisms are discussed in relation to habitat choice and behaviour.

Key words: Adhesion, adhesive gland system, glue compounds, histochemistry, *Euprymna*, *Idiosepius*, protein-polysaccharide complex

INTRODUCTION

Attachment in cephalopods is primarily achieved by reduced pressure systems as in suckers on the arms, tentacles or defined dermal structures on the mantle and tentacles (Muntz and Wentworth, 1995; von Boletzky and Roeleveld, 2000). Two genera of cephalopods (*Euprymna*, Sepiolidae; *Idiosepius*, Idiosepiidae) produce glue in adhesive glands, also termed adhesive organ (Nesis, 1982; Norman, 2003).

Euprymna scolopes live in near-shore benthal habitats and hide during the day in the sediment (Moynihan, 2002). The animals secrete glue to coat themselves totally with sand.

In case of danger they release sand instantaneously to deflect predators (Singley, 1983; Shears, 1988). Ultrastructural and histochemical examinations show that *Euprymna scolopes* has goblet and ovate cells in a duo-gland adhesive system all over the body (Singley, 1982). Between these cells are non-secretory interstitial cells (Fig. 1 and 2). The goblet cells contain large, electron dense granules (neutral mucopolysaccharides), responsible for adhesion. The finely granular secretory material of the ovate cells, located in a large vesicle appears to be basic proteins. During secretion these proteins transform to highly sulphated acidic proteins. Singley (1982) assumes that the acidic mucoproteins cause de-adhesion.

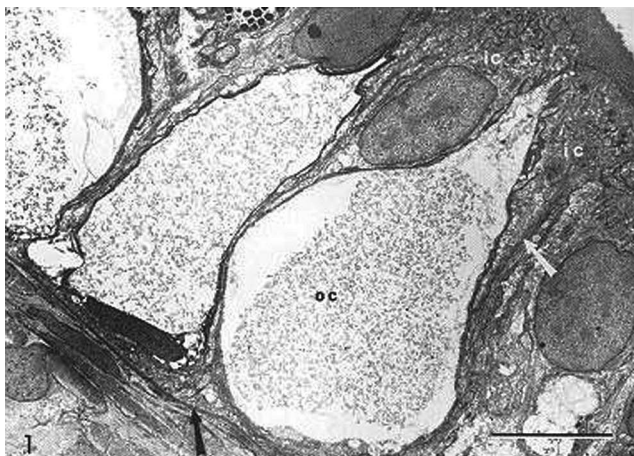


Fig. 1 Cross section of dorsal epithelium at *Euprymna scolopes*. Sac-like ovate cells (oc) with fine granular material occur in the adhesive organ, presumably responsible for de-adhesion. Interstitial cells (ic) possess intracellular filaments (white arrow). Scale bar = 10 μm Figure from Singley (1982).

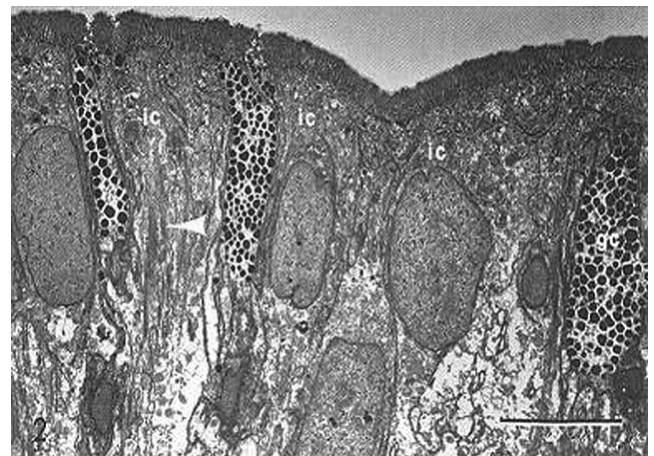


Fig. 2 Goblet cells (gc) have a long, tube-shaped form and are filled with large granules. Its secretion product is responsible for adhesion at *Euprymna scolopes*. The white pointer marks the intracellular filament of the interstitial cells (ic). Scale bar = 10 μm Figure from Singley (1982).

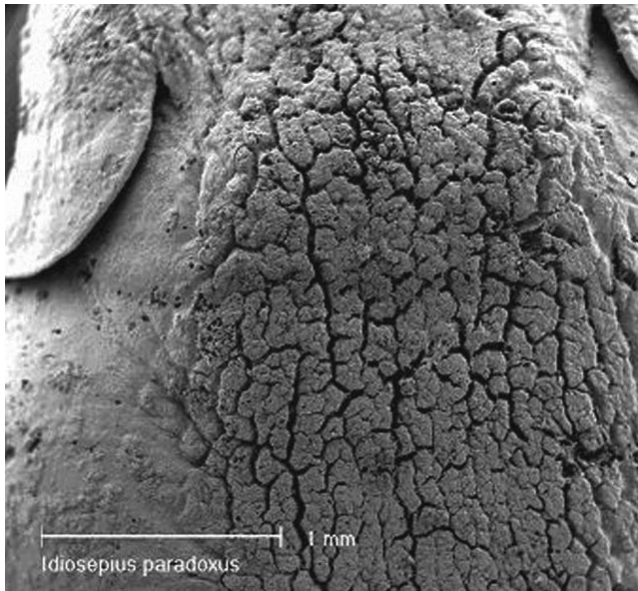


Fig. 3 SEM image of the adhesive organ of *Idiosepius paradoxus*. Sasaki (1921, p. 210) describes the adhesive organ as follows: “It is represented by a longitudinal corrugated area extending along the posterior three-fourths of the back. The folds run quite irregularly without any definite mode of arrangement, and show also fine furrows and pits”.

Idiosepius lives in near-shore shallow waters between sea grass and mangrove area. It camouflages during the day, sticking to the underside of sea grass leaves or algae (Moynihan, 1983; Hylleberg and Nateewathana, 1991; Jackson, 1992). Hiding there, the animals wait to capture prey swimming by, and females adhere also for spawning (Natsukari, 1970; Jackson, 1992; Lewis and Choat, 1993; Kasugai, 2000; Kasugai, 2001). In contrast to *Euprymna*, the adhesive organ of *Idiosepius* is restricted to the posterior part of the fin region of the dorsal mantle side (Fig. 3).

Previous results of Sasaki (1921) indicate that five different cell types can be distinguished histologically in the adhesive organ of *Idiosepius paradoxus*, namely columnar cells, granular cells, goblet cells, interstitial cells and basal cells. He assumes that the columnar cells are responsible for adhesion whereas the interstitial cells work pressure-induced and stimulate the secretion of adhesive substances from the columnar cells. No information is available on the function of the granular cells, goblet cells and basal cells or on the de-adhesion mechanisms (Sasaki, 1921).

MATERIAL AND METHODS

Histological, histochemical and ultrastructural methods were applied to elucidate the nature of the secretion in the epithelial cells of three *Idiosepius* species (*I. biserialis*, *I. paradoxus* and *I. pygmaeus*). For light microscope examinations, mantle tissue samples were fixed in two different acetic-alcohol-formalin mixtures (AAF I Lillie, 1949 and AAF II Böck, 1989) or for 1 h at room temperature in Carnoy solution (Kiernan, 1999); embedded in

paraffin (melting point 51–53 °C), cut in 7 µm sections, mounted on glass slides and dried at room temperature before use.

For ultrastructural investigations, specimen of *Idiosepius paradoxus* were fixed in 70% EtOH, dehydrated in a graded series of ethanol, washed several times in acetone, dried with HMDS (Hexamethyldisilazane) mounted on stubs, coated with gold in a Polaron 5800 sputter coater and examined using a Philips XL 20 scanning electron microscope.

Following Singley’s (1982) methods on *Euprymna scolopes* several histological and histochemical tests were employed to elucidate the nature of the epithelial secretions of *Idiosepius*.

The trichrome method **AZAN** (Heidenhain, 1905) was used to provide an overview of the glandular system and structural details. The periodic acid-Schiff (**PAS**) method (McManus and Mowry, 1960) was used to detect neutral hexose sugars units in the adhesive organ.

To differentiate between neutral and acidic mucosubstances in the adhesive organ, the following staining methods were used: the periodic acid-diamine method (**PAD**) according to Spicer and Jarrels, 1961 and Spicer, 1965 for 7, 24 and 48 h at pH 4.0; alcian blue 8GX (**AB**) (McManus and Mowry, 1960) at pH 1.0 and 2.5 (for 2 h at 20 °C) combination with PAS; azure A (**AA**) (Spicer, 1960) in different buffers (HCl-phosphate or phosphate-citrate) at graded pH levels (30 min in pH 0.5; 1.0 and 3.2); toluidine blue (**TB**), 0.1% in 30% ethanol for 20 min (Kramer and Windrum, 1954).

Proteins were detected with Biebrich Scarlet (**BS**) for 1 h at 20 °C (0.04% BS in phosphate buffer) at pH 6.0 (Spicer and Lillie, 1961) and at pH 8.0, 9.5 and 10.5 in Laskey’s glycine buffer (McManus and Mowry, 1960) and Fast Green FCF (**FG**) (0.1% FG for 30 min at pH 8.1) (Böck, 1989).

Key to Abbreviations

AA	Azure A	HMDS	Hexamethyldisilazane
AAF	Acetic-Alcohol- -Formalin fixative	ML	Mantle length
AB	Alcian Blue 8GX	PAD	Periodic acid- -p-Diamine
BS	Biebrich Scarlet	PAS	Periodic acid-Schiff
FG	Fast Green	TB	Toluidine Blue O

RESULTS

The adhesive organ in all *Idiosepius* species can be distinguished easily from the remaining body epithelium (30 µm in *Idiosepius biserialis*, 40 µm in *Idiosepius pygmaeus*) by its greater thickness (60–80 µm in *Idiosepius biserialis* and 80–100 µm in *Idiosepius pygmaeus*).

The adhesive organ consists of five different cell types (Fig. 4) (columnar, granular, goblet, interstitial and basal cells), which can be distinguished morphologically and on account of their chemistry of their secretion (von Byern et al., 2005; Cyran, von Byern, and Klepal, 2005):

Columnar cells are pear-shaped and tapering towards the surface; the cells are densely filled with fine granules (1 µm in diameter). Granular cells are oblong and tube-shaped and contain large spherical to polygonal granules (Fig. 5) (3–5 µm in diameter) of uniform density. Gob-

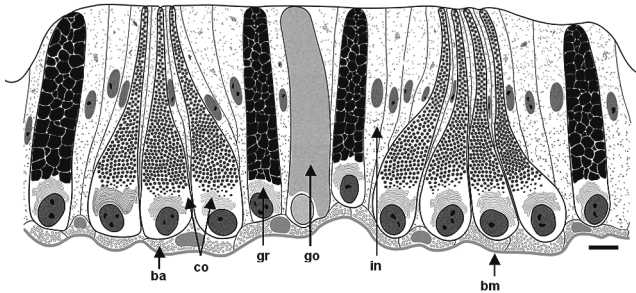


Fig. 4 Schematic drawing of the adhesive organ of *Idiosepius* with its characteristic cell types: bm-basal membrane, co-columnar cells, gr-granular cells, go-goblet cells, in-interstitial and ba-basal cells. Scale bar = 2 μ m Drawing by N. Cyran, et al., 2005.

let cells are round to sac-shaped, tapering towards their apical ends; its secretory material is finely granular. Interstitial cells between the secretory cells are long and slender; the cells are free of secretory material. Basal cells line the basal membrane and contain vacuoles of uniform density.

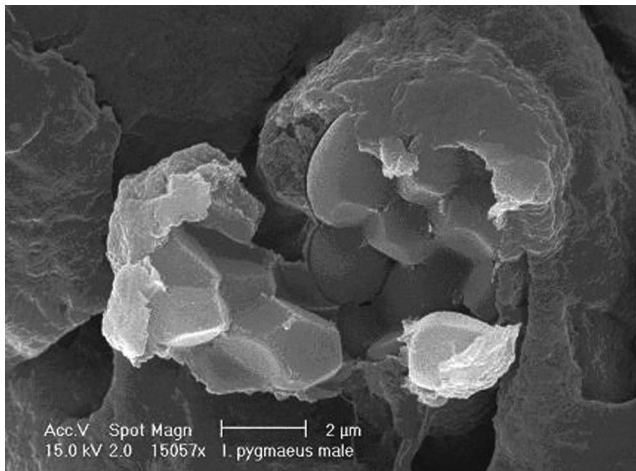


Fig. 5 The granular cell type with larger polygonal granules, packed tightly together.

Histochemical tests (Fig. 6a and b) show that the three glandular cell types (columnar, granular and goblet cells) are periodate-reactive (PAS) and moderately reactive with PAD. No γ -metachromasia is effected with Toluidine Blue and Alcian Blue, demonstrating acid groups. Biebrich Scarlet at all pH levels and Fast Green show weak (columnar cells), positive (granular cells) to strong positive (goblet cells) reactions for basic proteins.

DISCUSSION

The histochemical results of *Idiosepius* indicate that the secretory material consists of a protein-polysaccharide complex. Anyhow, the ratio of protein and polysaccharide varies strongly between the cells. Columnar cells contain a high proportion of

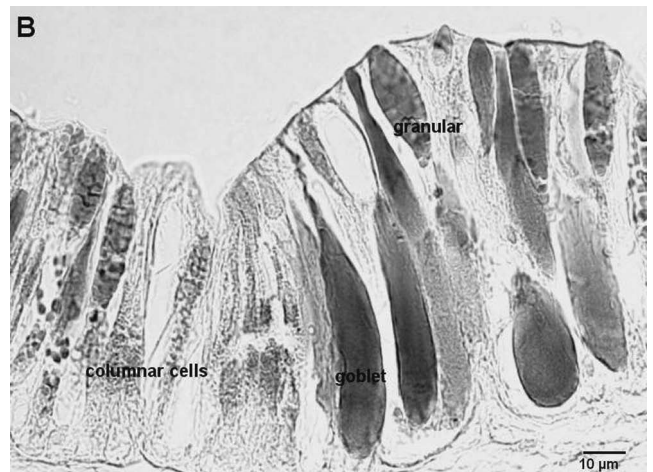
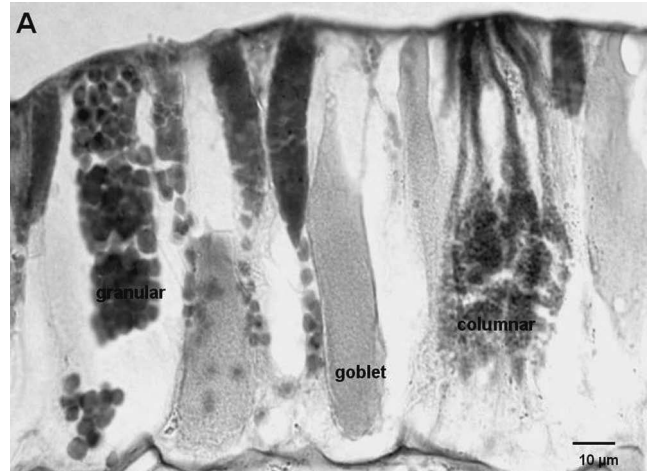


Fig. 6 Histochemical reactions of the cell types in the adhesive organ of *Idiosepius*. A. PAS with glutaraldehyde fixation, B. Biebrich scarlet. Interstitial and basal cells don't react with any of the applied histochemical tests.

sugar and few fractions of proteins. Granular cells have a balanced ratio of sugar and proteins, while the goblet cells have a higher proportion of proteins and a lower fraction of sugar units. Acidic substances are absent in all *Idiosepius* species.

In comparison with *Euprymna scolopes* (Singley, 1982) the adhesive organ in *Idiosepius* shows similarities and differences in the morphology and secretory components of the glandular cell types¹ (Despite the morphological similarities of the cell types in the two genera we follow the terminology used by Sasaki (1921) (see also Packard, 1988; Budelmann, Schipp, and von Boletzky, 1997). The goblet cells of *Euprymna* resemble the granular cells of *Idiosepius* and contain both neutral hexose sugars. The ovate cells of *Euprymna* correspond morphologically to the goblet cells of *Idiosepius*. Anyhow the basic proteins in this cell type in *Euprymna* become highly acidic during secretion (Singley, 1982). On the contrary, a change of the associated protein-polysaccharide complex to an acidic complex in this cell type can be excluded for *Idiosepius*. The appropriate tests (AB, AA, TB) were negative, even on secreting cells. The interstitial cells of *Euprymna scolopes*, *Idiosepius biserialis* and *Idiosepius pygmaeus* do not show any histochemical reaction and are

¹ Despite the morphological similarities of the cell types in the two genera we follow the terminology used by Sasaki (1921) (see also Packard, 1988; Budelmann, Schipp and von Boletzky, 1997).

presumably not involved in the secretion of mucous substances for adhesion or de-adhesion. The columnar cells and basal cells are restricted to the adhesive organ of *Idiosepius*.

The histochemical nature of the secretory products suggests that adhesion and de-adhesion is not evoked by a duo-gland adhesive system such found as that in *Euprymna scolopes* (Singley, 1982) and gastropods (Grenon and Walker, 1980; Shirbhate and Cook, 1987). Adhesion in *Idiosepius* is rather effected by a mechanism as described by Stefan (1874).

This form of adhesion involves the presence of two flat surfaces with a thin film of liquid in between. Stefan's formula predicts an increase of adhesion force by increased viscosity of the liquid between two flat surfaces (Stefan, 1874). Such a form of adhesion, induced by highly viscous mucus secreted from the pedal glands, can be found in many gastropods (Grenon and Walker, 1978).

The different adhesive substances and mechanisms of de-adhesion in these two squid taxa serve different purposes. They can be explained as ecological and behavioural adaptations. *Euprymna scolopes* uses the glue to cover itself with a coat of soft sediment (sand, mud) and disconnects from it fast and over the whole body. *Idiosepius* attaches with a small adhesive area to different substrates (seaweeds, seagrass leaves, roots). An easy but slow disconnection is effected without special de-adhesive substance but presumably by a mechanical or dissolving mechanism.

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