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Morphology of the Dart and the Dart Sac in the Land Snail *Nesiohelix samarangae*

In-Seon Byun, Yong-Hun Jo, Yong-Seok Lee and Kye-Heon Jeong

Department of Biology, College of Natural Sciences, Soonchunhyang University, Asan, 336-745, Korea

ABSTRACT

An anatomical and ultrastructural study on the dart sac and the dart of a Korean snail *Nesiohelix samarangae* was conducted to understand their morphological characteristics with the help of the light microscope and electron microscopes, TEM and SEM. *Nesiolelix samarangae* had two darts which are pure white structures 6-8 mm in length, tapering from 0.6-0.8 mm to 0.15-0.3 mm. The dart sac had a long conic lumen subdivided into two by a septal wall, and the darts were centrally embedded in the thick muscular layers of the sac. The darts occupied each of the two luminal spaces one per each.

The convexed surfaces of the darts had many crystal buds in the shape of the petals. Otherwise, the convexed surfaces of the darts had numerous crystal buds in the shape of candle or topaz.

The luminal surface of the dart sac was covered with a single columnar epithelium. The epithelial cells possessed microvilli on their free surface.

Keywords: Dart, Dart sac, Tubercle, Crystal buds.

INTRODUCTION

The land snail has a dart sac complex which is an auxiliary organs of the reproductive system. The dart sac complex consists of three parts; a dart sac, an accessory sac, and a mucous gland. The dart sac is an appendix organ joining the vagina at its end with the muscular penis in which they open together into the common genital atrium (El-Sherief *et al.*, 1995).

The dart sac has one or several calcified darts. The dart is an elaborate $CaCO_3$ structure found in the reproductive system of many terrestrial snails (Dillaman, 1981). The calcium content of the dart and dart sac was measured to determine the magnitude of $CaCO_3$ deposition during dart formation (Dillaman, 1981). Tompa and Wilber (1982) monitored the dart formation in *Helix aspersa* by X-radiography. Early authors (Tompa and Wilbur, 1982) noticed that some species lost their dart in mating and new one formed within few days. Tompa (1980) examined the structure composition and biological function of the dart of a slug *Philomycus carolinianus.*

Chung (1987), Koene and Chase (1998a, b), and Adamo and Chase (1988) observed the courtship, copulation and dart shooting behavior of some land snails. Landolfa *et al.* (2001) suggested possibilities of the function of the dart and dart shooting behavior. The function of the dart and dart sac is still uncertain. Hunt (1979) and Dillaman (1981) observed the ultrastructures of the dart with scanning electron microscopes to understand the reproductive system and mating behavior.

This study was carried out to find out the morphology and ultrastructures of the dart and dart sac of *Nesiohelix samarangae*, a Korean land snail.

MATERIALS AND METHODS

Several individuals of the Korean land snail, were collected from a small island, Gauido, located in the West Sea of Taean-gun, Chungnam, Korea. The snails

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Corresponding author: Jeong, Kye-Heon
Tel: (82) 41-530-1249 e-mail: jngkh@asan.sch.ac.kr
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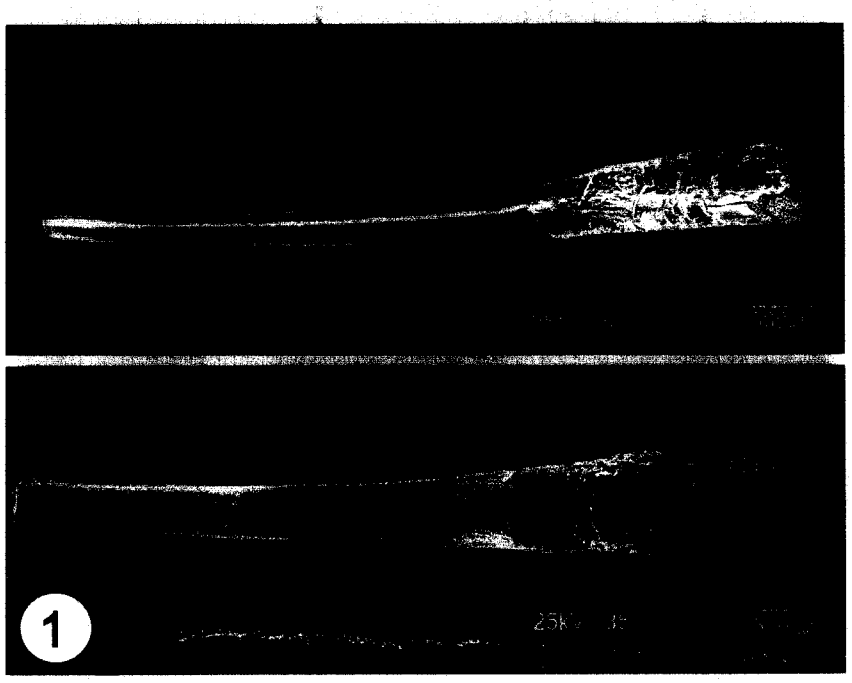


Fig. 1. Scanning electron micrographs of the two darts removed from the dart sac of a snail. The one (A) is thinner and smaller than the another one (B).

Fig. 2. A model showing the darts and dart sac. The luminal cavity of the dart sac is subdivided into two along its length by a septal wall. The darts occupy the subdivided lumen one per each. (Cc: concaved surface of the dart, Cv: convexed surface of the dart, D: dart, DS: dart sac, Cl cleft of the dart, SW: septal wall)

were kept in plastic containers at 25℃ and fed lettuce, carrots and calcium carbonate powder.

For light microscopic observations, dart sacs were dissected from the snails, then fixed with 10% neutral buffered formalin for 24 hours, placed into a mixture of 5% formalin, potassium acetate, and concentrated nitric acid, a decalcifying medium to remove the dart. The specimens were washed with 70% ethyl alcohol, dehydrated, embedded in paraffin, and sectioned at 7 μm in thickness. The tissue sections were stained with methylene blue and hematoxylin-eosin, and examined with a light microscope, Optiphot-II (Nikon).

For transmission electron microscopic observations, dart sacs were dissected from the snails, prefixed with 2% glutaraldehyde for 2 hours, and postfixed with 2% OsO_4 for one and half hours. After removing the darts, the specimens were washed with 70% ethyl alcohol, dehydrated in alcohol series, embedded in spur, sectioned 70 nm in thickness with a

ultramicrotome (Reichert supernova ultramicrotome). The thin tissue sections were double stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope (JEM-100CX II).

For scanning electron microscopic observations, the dart sac was immersed in 10% KOH solution for overnight to dissolve its tissue part except the darts. The darts isolated and rinsed with double distilled water were air dried. The dried darts were coated with gold particle 200 nm in thickness and examined with a scanning electron microscope (JSM-5410LV).

RESULTS

1. The darts

The land snail *Nesiohelix samarangae* had two darts (Fig. 1). The darts were embedded in a long conical muscular layers of the dart sac (Fig. 2). The luminal cavity of the dart sac was subdivided into two along its length. The darts were pure white structures 6-8 mm in length. The one is thinner and shorter than the other one (Fig. 1).

The darts tapered from the base to the tip looked like compressed cones (Fig. 1). Thus, they had a convexed surface and a concaved surface which showed different matters of sculpture. The two darts faced their concaved surfaces toward the subdividing septal wall of the dart sac. The concaved surfaces had many crystal buds in the shape of the petals (Figs. 6, 7). Otherwise, the convexed surfaces of the darts had numerous crystal buds in the shape of a candle or topaz (Fig. 8, 9).

The darts were completely dissolved in a mixture of 5% formalin, potassium acetate, and concentrated nitric acid, a decalcifying medium.

2. The dart sac

The dart sac of *Nesiohelix samarangae* was a small club like protuberant organ. The luminal cavity of the dart sac was subdivided into two along its length (Fig. 2).

The cross section taken through the basal portion of the dart sac showed two tubercles in the lumen (Fig. 10, 11). The tubercles had lots of secretory granules (Fig. 12, 13). The inner surfaces of the dart sac were covered with single columnar epithelium. The epithelial cells had microvilli on their luminal surfaces (Fig. 14, 15). The luminal spaces of the dart sac were filled with the fully grown darts so that the dart surfaces and the epithelial cells of the dart sac were in contact (Fig. 2, 3).

DISCUSSION

The dart is an elaborate $CaCO_3$ structure found in the reproductive system of many terrestrial snails (Dillaman, 1981). Snails have wide variety of shapes, form and sizes and the number of the darts. Snails also have variety of shapes, form and sizes of dart sac depending on the variety of the dart (Adamo and Chase, 1998; Dillaman, 1981; Tompa and Wilbur, 1982).

The importance of the dart in the mating behavior is uncertain and the role of the dart has been controversy.

In *Helix aspersa* mating can be divided into three distinct phases; introductory behavior, dart shooting, and copulation (Adamo and Chase, 1988; Chung, 1987; Leonard, 1992). The dart is pushed and thrust against the body of a mating partner and eventually wind up (usually) penetrating the body wall, especially on the right side of the recipient's foot (Tompa, 1980). But, such as *Philomycus, Ventridens, Parmarion* don't lose their dart during mating. When expelled, the dart is covered with thick mucus (Koene and Chase, 1998). In *Helix aspersa*, after the mating they regenerate a new dart in about one week (Tompa and Wilbur, 1982). Dart formation begins at the tip of a tubercle where a small group of epithelial cells (Dillaman, 1981).

The dart initiates at the tip of the tubercle, first appearing as a small $CaCO_3$ cone. The secretory activity of the tubercle started at the tip and then proceeded posteriorly until the entire tubercle is active. Such a pattern of activity would provide a driving force for the forward movement of the dart in the dart sac and explain the increasing circumference of the hollow center (Dillaman, 1981). He suggested that the mineralization in the dart follows a pattern based on an interplay between a calcifying and non-calcifying secretion, the former arising from the

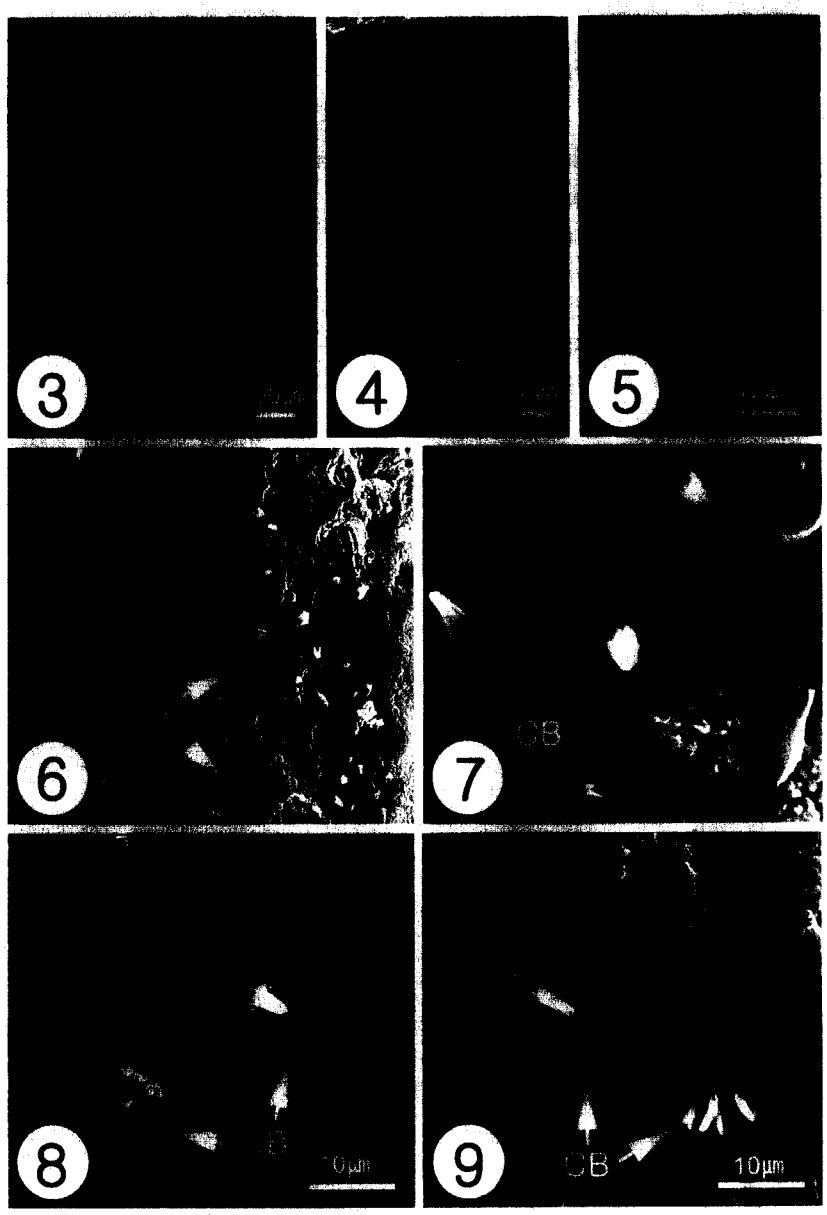


Fig. 3. Scanning electron micrograph of the dart sac crossly sectioned. The concaved surfaces of the two darts faced to each other. The septal wall is invisible because of a damage. (D: dart, DS dart sac)

Fig. 4. Scanning electron micrographs of the dart fractured. (CcL: concaved surface layer of the dart, CvL: convexed surface layer of the dart, Cl: cleft of the dart)

Fig. 5. An enlarged view of the calcified convexed layer. (CvL: convexed surface layer of the dart, Cl: cleft of the dart)

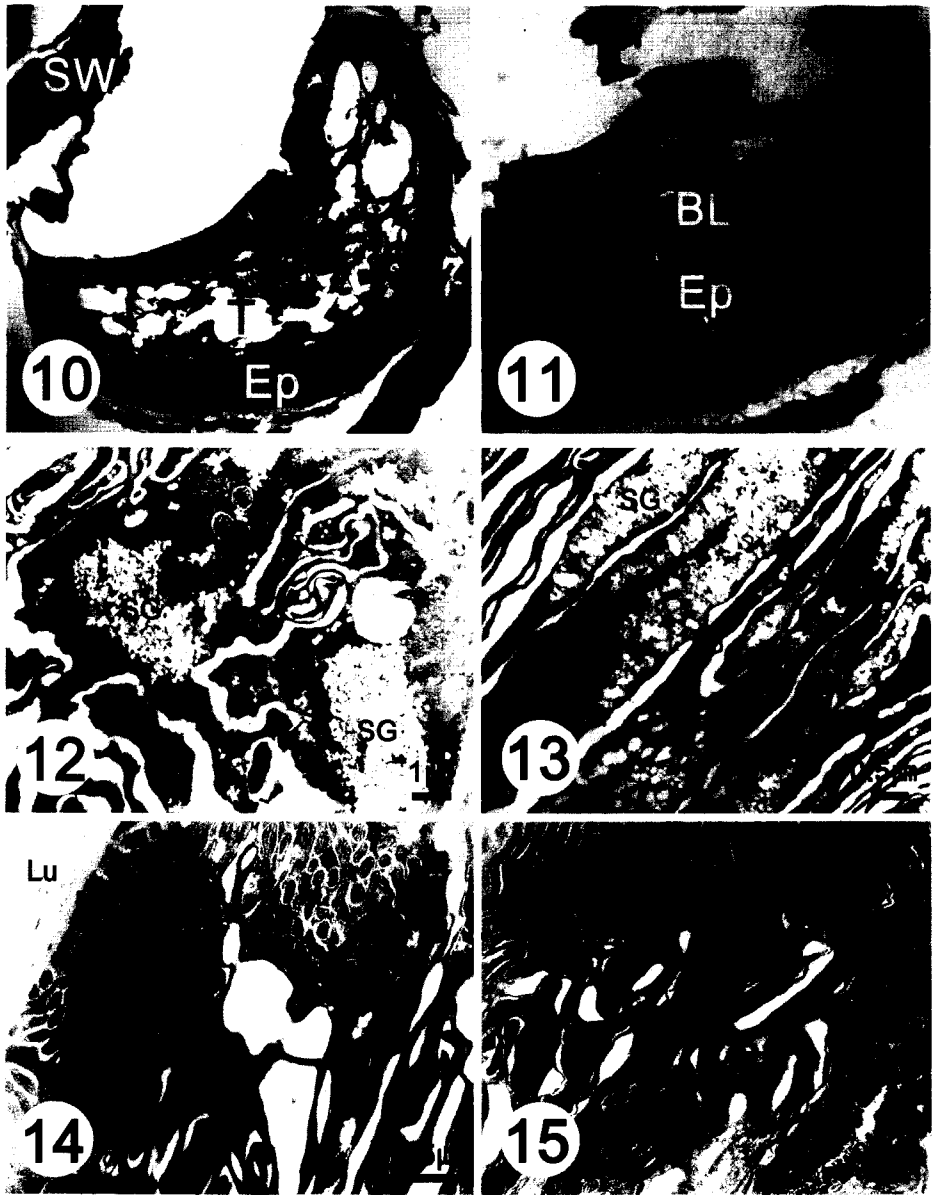
Figs. 6-7. The crystal buds (arrow) in the shape of the petals on the concaved surface of the dart. (CB: crystal buds)

Fig. 8. The crystal buds (arrow) in the shape of a candle on the convexed surface of the dart. (CB: crystal buds)

Fig. 9. The crystal buds (arrow) in the shape of the topaz on the convexed surface of the dart. (CB: crystal buds)

epithelium of the dart sac, and the latter from the tubercle epithelium. Hunt (1979) described these two types of protein, namely, the matrix protein and the coating protein correspond calcifying and non-calcifying organic material.

The dart of *Nesiohelix samarangae* was completely



Figs. 10-11. Light micrographs of the tubercle stained with hematoxylin-eosin. Fig. 10: × 100, Fig. 11: × 400 (BL: basal layer of the tubercle, Ep: Epithelium of the tubercle, SW: septal wall, T tubercle)

Figs. 12-13. Transmission electron micrographs of the tubercle. The epithelial cells of the tubercle contains secretory granules. (SG: secretory granules)

Figs. 14-15. Transmission electron micrographs of the dart sac epithelium. The epithelial cells of the middle part of the dart sac have numerous mitochondria. (Lu: dart removed lumen, Mv: microvilli, M: mitochondria)

dissolved in a mixture of 5% formalin, potassium acetate, and concentrated nitric acid, a decalcifying medium, suggesting that the dart is a calcified structure as in usual.

In *Nesiohelix samarangae*, the basic structure of the dart was very different from those of the *Helix aspersa* and *Helix pomatia*. *Nesiohelix samarangae* had the petals, candle, and topaz like crystal buds on the surface of the dart.

The tubercles of *Nesiohelix samarangae* had lots of secretory granules. The tubercle secretion and subsequent calcification at the base of the dart would push the forming dart forward like previously reported (Dillaman, 1981).

Having two tubercles in *Nesiohelix samarangae* seems that each of the darts grow independently in the subdivided lumen of the dart sac.

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