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Histology of the androgenic gland in *Eriphia verrucosa* (Forskål, 1775) (Decapoda, Brachyura)

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Abstract: This study investigated the histology of the androgenic gland in *Eriphia verrucosa* (Forskål, 1775). The results obtained with light microscopy show that the androgenic gland in this species consists of 2 cell types. Type 1 cells are small and do not contain a secretory product. Type 2 cells are considerably larger and may produce a proteinaceous secretion. This study identified the location and structure of the androgenic gland in *Eriphia verrucosa*, which is an economically important brachyuran crab, and revealed the chemical nature of the secretions produced by its cells.

Key words: Decapoda, crab, *Eriphia verrucosa*, androgenic gland

Eriphia verrocosa (Forskål, 1775) (Decapoda, Brachyura)'da androjenik bezin histolojisi

Özet: Bu çalışmada *Eriphia verrucosa* (Forskål, 1775)'nın androjenik bezi histolojik olarak incelenmiştir. Işık mikroskobu çalışmalarından elde edilen sonuçlar bu türde androjenik bezin iki tip hücreden oluştuğunu göstermiştir. Birinci tip hücreler küçük ve salgı ürünü içermemektedir. Ikinci tip hücreler oldukça büyüktür ve protein karakterli salgı üretebilmektedirler. Bu çalışmanın sonuçları, ekonomik açıdan önemli bir brachyuran yengeci olan *Eriphia verucosa*'da androjenik bezin bulunduğu yeri, yapısını ve androjenik bezleri oluşturan hücrelerin salgılarının kimyasal içeriği belirlenmiştir.

Anahtar sözcükler: Dekapoda, yengeç, *Eriphia verrucosa*, androjenik bez

Introduction

Sexual differentiation in the male reproductive system of crustaceans is not controlled by the gonad, but by the androgenic gland (Charniaux-Cotton, 1954; Hasegawa et al., 1993; Taketomi et al., 1996; Cui et al., 2005; Sagi and Aflalo, 2005; Barki et al., 2006). As reported by Charniaux-Cotton and Payen (1985), and Minagawa et al. (1994), the androgenic gland secretes an androgenic hormone that plays a key role in the regulation of male sexual differentiation. The releasing type of androgenic gland is holocrine (Charniaux-Cotton, 1962), and it is commonly reported that the nature of androgenic hormone in decapod crustaceans is protein or polypeptide (King,

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1964; Sun et al., 2000). The morphological and physiological effects of the androgenic gland on decapod crustaceans have always been topics of concern (Taketomi et al., 1990; Taketomi et al., 1996; Sagi et al., 1996; Fowler and Leonard, 1999; Khalaila et al., 1999). Studies that have isolated androgenic hormone and reported that it consists of glycosylated protein are very significant (Okuno et al., 1999). Although the function of the androgenic gland has been well demonstrated by such studies as those cited above, the number of studies on the structure of the androgenic gland and the nature of its secretory products in brachyuran crabs is lacking (Awari and Kiran, 1999). As such, it was deemed worthwhile to determine the location and histology of the androgenic gland, and the nature of androgenic hormone in *Eriphia verrucosa* (Forskål, 1775), a brachyuran crab species that is commonly eaten by humans. This study could also serve as a basis for future aquacultural, biotechnological, and developmental research on brachyuran crabs.

Materials and methods

Twelve adult male *Eriphia verrucosa* (mean carapace length: 80 mm) specimens were collected from the Bay of Karaburun (Black Sea) in Turkey at depths of 1-5 m. Once in the laboratory, the animals were killed by cold treatment (5 min at −20 °C). Lifting the dorsal carapace, the androgenic gland was dissected under a stereomicroscope, fixed in Bouin's solution for 12 h at 20 °C, and placed in 70% ethanol. The tissues were serially dehydrated in an ethanol series, cleared in xylene, and finally embedded in paraffin. Sections 5-µm thick were cut, placed on microscope slides, and stained with hematoxylin-eosin for histological observation, and stained with bromphenol blue, periodic acid-Schiff, Alcian blue, and aldehyde fuchsin for histochemical analysis under light microscopy (Pearse, 1960; Bancroft and Stevens, 1982). The diameter of androgenic gland cells (type 1 and type 2 cells) was measured with an ocular micrometer in 5 random visual fields corresponding to 120 cells counted in each histological section.

Results

The androgenic gland in *Eriphia verrucosa* is attached to the posterior vas deferens, forming a cord, and is covered with connective tissue (Figure 1A). The cells are located at an external position on the wall of the vas deferens that faces the hemocoel surface of the duct.

The androgenic gland consists primarily of 2 types of cells, which in *Eriphia verrucosa* are referred to as type 1 and type 2 (Figure 1B). Type 1 cells are small, have a round nucleus, and are characterized by a high nucleocytoplasmic rate. The minimum size of type 1 cells is 7.5 µm and maximum size is 11 µm. The cytoplasm does not stain with bromphenol blue, and gives Alcian blue, aldehyde fuchsin, and PAS negative reactions. Type 2 cells are considerably larger, with an elliptical nucleus. The minimum size of type 2 cells is 12.5 µm and the maximum size is 27.5 µm. The cytoplasm lightly stains with hematoxylin. They produce and accumulate a secretory product in the cytoplasm. After type 2 cells release their secretory product vacuolization occurs in the cytoplasm and histologically this area has the appearance of a holocrine gland (Figure 1B). This product in the cells stain with Bromphenol blue at different densities, while giving a negative reaction to Alcian blue and aldehyde fuchsin (Figure 1C). The secretory product in type 2 cells, which is mature and stains deeply with Bromphenol blue, gives a PAS-positive reaction, while the secretory product in the early maturation stage gives a PAS-negative reaction (Figure 1D).

Discussion

The androgenic gland was first reported by Cronin (1947) and Charniaux-Cotton (1954) carried out the first experimental study that explained the role of the androgenic gland in the amphipod *Orchestia gamarella* (Guérin, 1825), naming this gland the androgenic gland in 1955. As reported for decapod crustaceans in previous studies, the androgenic gland in *Eriphia verrucosa* is located in the posterior vas deferens; however, the arrangement of cells in the androgenic gland is variable. These cells might form simple strands, as in most brachyurans, lobules as in Majoidea, or cords as in penaeids. In addition, they sometimes show anastomosis (Charniaux-Cotton and Payen, 1985; Fingerman, 1992); however, they all are covered with connective tissue externally. These characteristics of the androgenic gland, which have

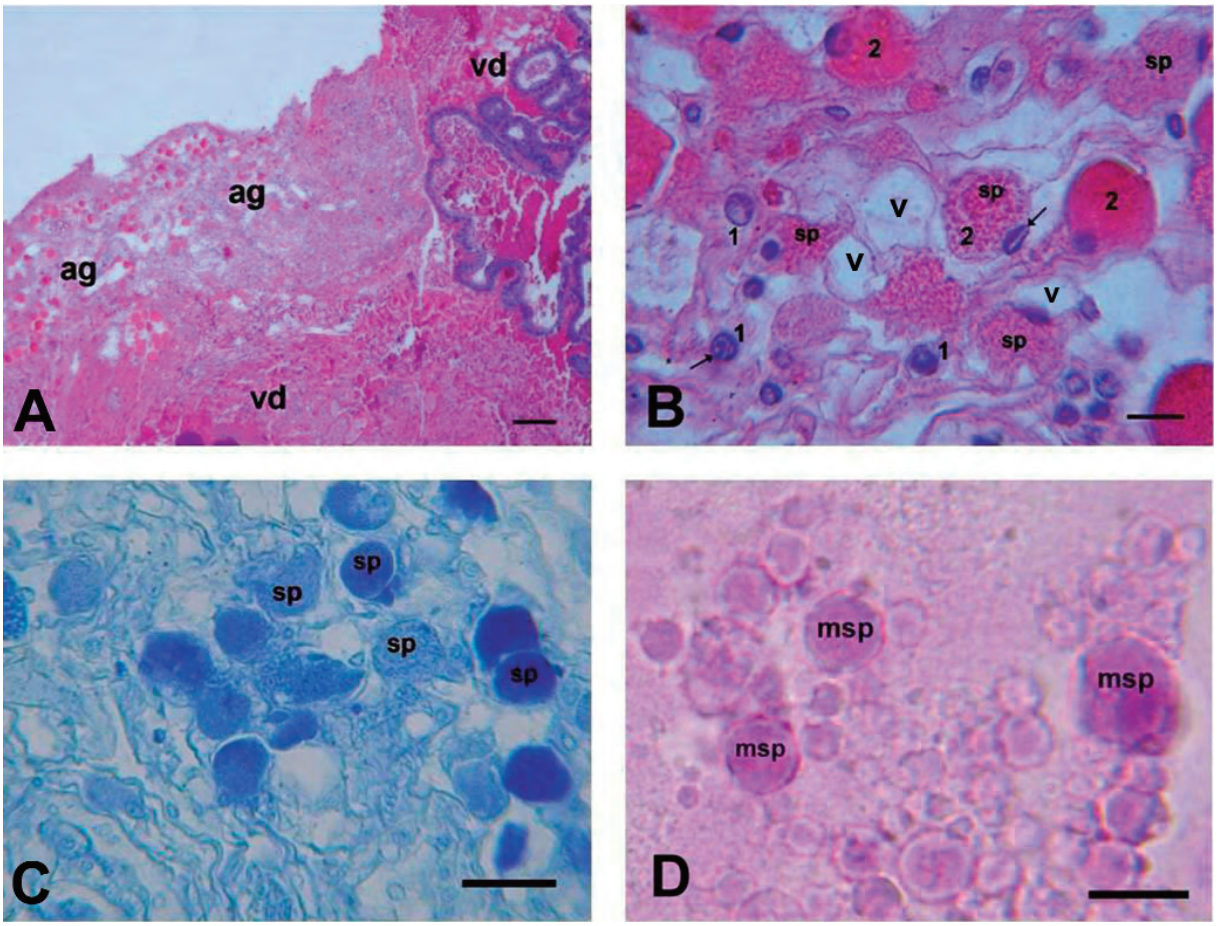

Figure 1. The androgenic gland in *Eriphia verrucosa*. A. The androgenic gland (ag) attached to the posterior vas deferens (vd) in an adult male *Eriphia verrucosa* stained with hematoxylin-eosin (scale bar: 300 µm). B. Type 1 cell (1) and type 2 cell (2) in the androgenic gland, nucleus (arrow), secretory product (sp), and vacuole (v) (scale bar: 10 µm). C. Secretory product (sp) of type 2 cells stained to different densities with Bromphenol blue (scale bar: 30 µm). D. Mature secretory product (msp) gives PAS-positive reaction in type 2 cells (10 µm).

been described above in general terms, are similar to each other in crab species (King, 1964; Thampy and John, 1970; Payen et al., 1971; Joshi and Khanna, 1987; Minagawa et al., 1994), prawns (Carlisle, 1959; Payen et al., 1982; Sagi and Aflalo, 2005), shrimps (Thampy and John, 1972, 1973; Campos-Ramos, 2006; Kim et al., 2006), and crayfish (Fowler and Leonard, 1999; Barki et al., 2006).

Hoffman (1969) reported that androgenic gland cells originate from the epithelium of the ejaculatory bulb, and that the androgenic gland activates during the winter and spring in the shrimp *Pandalus platyceros* (Brandt, 1851). He divided the development of the androgenic gland into 6 stages in a study on *Pandalus platyceros*, and reported that vacuolization increases significantly and secretory activity is high during the third stage (Foulks and Hoffman, 1974). In type 2 cells in the androgenic gland in *Eriphia verrucosa*, vacuoles are mostly observed after high secretion activity during the mating season. There are significant differences in the activation and release of secretory material according to season (Carpenter and DeRoos, 1970; Liu et al., 2008). The androgenic gland of the crab *Scylla paramamosain* (Estampador, 1949) is largest during the major mating season (July-September) (Liu et al., 2008). The major mating season of *Eriphia verrucosa* is between late July and the end of August (Erkan et al., 2008), which corresponded to the destruction of type 2 cells after releasing their contents.

While Fingerman (1992) reported that the cells that make up the androgenic gland might be different in brachyuran crabs, as in other crustacean groups, Thampy and John (1970) reported that the androgenic gland in *Ocypoda platytarsis* (H. M. Edwards, 1852) consists of 3 different types of cells. The androgenic gland in the crayfish *Procambarus clarkii* (Girard, 1852) was reported to consist of 2 different types of cells (Taketomi, 1986). While Awari and Kiran (1999) reported that 3 different types of cells make up the androgenic gland of *Macrobrachium rosenbergii* (De Man, 1879), a shrimp species, Okumura and Hara (2004) reported 2 different types for the same species. According to the histological findings of the present study, 2 cell types that differ from each other, at least in terms of size, were observed. Type 1 cells are considerably smaller than type 2 cells and can be distinguished easily from type 2 cells by their high nucleoplasmic rate; additionally, they do not contain secretory granules. On the other hand, type 2 cells can be distinguished very easily from type 1 cells by their elliptical nuclei and the secretory product that accumulates in their cytoplasm. It is thought that the variation in androgenic gland cell types might stem from the changes in the different stages of the secretory cycle of these cells (Thampy and John, 1970). Carpenter and De Roos (1970) reported that the structure of the androgenic gland in *Orconectes nais* (Faxon, 1885) varies according to the sexual activity or inactivity of each individual. We think that detailed seasonal research might be necessary in order to precisely determine if the androgenic gland cell types are in fact different, and if the variation in cell types is related to different stages of the secretory cycle of the androgenic gland or to the sexual activity or inactivity of individuals.

Fingerman (1992) reported that there is no consensus among researchers concerning the chemical structure of androgenic hormone in crustaceans. Ferezou et al. (1977) observed terpenoids (farnesyl acetone and hexahydro farnesyl acetone) in the androgenic gland in the crab *Carcinus meanas* (Linnaeus, 1758). Berreur-Bonnenfant et al. (1973) purified a lipoidal substance from the androgenic gland of the same species and reported that the purified substance inhibits vitellogenesis when injected into *Orchestia gamarella*. In addition, this substance induced secondary male sexual characteristics in *Talitrus saltatus* (Montagu, 1808) females (Sagi and Khalaila, 2001). It was reported that the secretion of the androgenic gland in *Macrobrachium rosenbergii*, a freshwater prawn, is steroidogenic (Veith and Malecha, 1983).

King (1964) reported that the androgenic hormone in *Pachygrapsus crassipes* (J. W. Randall, 1840) is proteinaceous. In his review, Sagi (1988) also reported that the androgenic hormone of decapods is proteinaceous. Miyawaki and Taketomi (1978), and Taketomi (1986) reported that the ultrastructural findings of their studies on *Procambarus clarkii*, a crayfish species, support the possibility that the androgenic gland hormone in this species is peptidergic-proteinaceous. Awari and Kiran (1999) reported that they found no evidence of the existence of lipids in the 3 cell types that make up the androgenic gland in *Macrobrachium rosenbergii*, and that their findings support the idea that the androgenic hormone is proteinaceous. In studies carried out by Sun et al. (2000) on the structure of the androgenic gland and the polypeptide profile of *Macrobrachium rosenbergii*, and by Zhang et al. (2000) on the separation and identification of the androgenic hormone of the same species, they both reported that this hormone is proteinaceous. The histochemical findings of the present study support that the secretory products of the androgenic gland are proteinaceous.

The monosex culture strategy has become common practice in fish-based aquaculture (Mires, 1977; Tayman and Shelton, 1978) and recent attempts have been made to apply this aquatechnology to crustacean culture, especially with economically important species (Curtis and Jones, 1995; Sagi et al., 1997). The production of monosex decapod populations is needed to elucidate the bioactive products of the androgenic gland that enable molecular and biochemical sex differentiation (Sagi and Aflalo, 2005).

To conclude, the present study identified the exact location of the androgenic gland in *Eriphia verrucosa* and observed that 1 of the 2 different cell types that make up the androgenic gland produces a proteinaceous secretion to which carbohydrate might be added just before it is released.

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