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Thoracic nerve cord-ganglia recognition in intraspecific and interspecific transplants in the white shrimp, *Penaeus (Litopenaeus) vannamei*

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ABSTRACT

The recognition response of *Penaeus (Litopenaeus) vannamei* against thoracic nerve cord–ganglia transplants from three species of Penaeidae: *P. vannamei, P. (Litopenaeus) stylirostris,* and *Trachypenaeus byrdi*, and one species of Palaemonidae: *Macrobrachium tenellum,* was evaluated. Implanted tissues were removed from recipients at the end of different incubation periods, catalogued based on three gross categories (missing, unmelanized, and melanized), and analyzed by histology. Some intraspecific implants of thoracic ganglia from *P. vannamei* were missing after a few weeks, without activating a haemocytic response leading to encapsulation. The same condition was observed for thoracic ganglia from *P. stylirostris* and *T. byrdi*. Unmelanized tissue sections from *P. vannamei, P. stylirostris,* and *T. byrdi,* were commonly recovered; however, most implants had a smaller size than their original length (5–6 mm). Intraspecific transplantation in *P. stylirostris* also generated a time-related reduction in graft size. Based on our observations, missing implants, small grafts and compacted structure of tissues indicate an absorption mechanism from the host without encapsulation.

Histological analysis of unmelanized tissues from Penaeidae reveals that the structure of nerve cords is similar to controls, showing living nerve cord cells surrounded by the neurilemma, and no haemocytic encapsulation at the periphery. Neurosecretory cells were also observed alive, but the structure of ganglia was different from control ganglia. On the other hand, tissues from *M. tenellum* were all encapsulated, showing distinctive black depositions and the distinctive layers of haemocytes. These findings suggest that neither interspecific nor intraspecific thoracic ganglia transplants within Penaeidae keep their tissue integrity inside *P. vannamei.* The endocrine implications for inducing ovarian maturation have to be investigated.

1. Introduction

The central nervous system of penaeoid shrimps is organized in an anterior brain or supraesophageal ganglion, circumesophageal connectives, a chain of thoracic ganglia and abdominal ganglia (Bell and Lightner, 1988). The sinus gland from eyestalks, the brain, and the thoracic ganglia have been considered as the source of a vitellogenesis stimulating hormone (VSH), which has not been characterized yet (Huberman, 2000). However, thoracic ganglia implants or extracts have produced increases in gonadal growth in shrimps, crabs, lobsters and crayfish (Otsu, 1960; Oyama, 1968; Hinsch and Bennett, 1979; Takayanagi et al., 1986; Quackenbush, 1986, for review; Fingerman, 1987, for review; Kulkarni et al., 1991). It was demonstrated that thoracic ganglion extracts from sexually active females of *Uca pugilator* induced precocious ovarian maturation in intact and eyestalk ablated crabs; extracts from sexually inactive females did not stimulate ovarian growth (Eastman-Reks and Fingerman, 1984).

Eyestalk ablation is the worldwide protocol for inducing ovarian maturation of cultured penaeoid species; however, alternatives to this procedure were considered a long-term goal for the industry (Quackenbush, 1991). Recently, a new protocol based on the repetitive injection of serotonin and spiperone was reported by Alfaro et al. (2004) for *Penaeus (Litopenaeus) stylirostris* and *P. (Litopenaeus) vannamei*, and serotonin was also evaluated in *P. monodon* by Wongprasert et al. (2006). Both studies were capable of inducing maturation and spawning at rates similar to eyestalk ablation.

The practical implications of using thoracic ganglia for inducing ovarian maturation and spawning of commercially important crustaceans such as penaeoid shrimps were evaluated by Hooi (1991) in *P. monodon*, injecting an intraspecific thoracic ganglia extract from spent spawners, without success. This author stated the importance of evaluating interspecific thoracic extracts from less valuable crustacean species. Yano et al. (1988) implanted thoracic ganglia sections of the lobster, *Homarus americanus* in *P. vannamei* recipients; however, tissue recognition was not evaluated and the authors reported some degree of ovarian maturation. The current knowledge on interspecific tissue recognition among crustaceans is limited, so there is no

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evidence to support the assumption that tissue from a lobster would be recognized as self by a shrimp (Vaca and Alfaro, 2000).

Research on tissue recognition in arthropod transplants indicates that a haemocytic response from the host will be activated depending on the phylogenetic closeness between donor and recipient species. In general, intraspecific transplants do not elicit encapsulation, but interspecific transplants provoke a strong haemocytic reaction; however, closely related species or genera may accept transplantation (Lackie, 1986, for review). In amphipod crustaceans it was established that hepatic caeca from *Gammarus pulex* implanted in *G. duebeni* provoke a strong encapsulation 24 h after surgery (Crompton, 1967). This author also found that intraspecific transplants of hepatic caeca in *G. pulex* only generate a haemocytic response at the cut surface of the tissue, where the connective tissue was damaged.

Encapsulation and nodule formation are the mechanisms triggered in arthropods as rejection against foreign tissues (Lackie, 1986). This haemocytic response consist of three concentric layers around the foreign tissue: necrotic, melanizing blood cells in the inner part, flattened cells in the middle and normally shaped cells in the outer region of the envelope. The three blood cell types (semigranular, granular, and hyaline cells) participate in the formation of the middle layer (Gotz, 1986).

The phenoloxidase activating system from granular and semigranular cells is responsible for the melanization of foreign objects in crustaceans including penaeoid shrimps (Ashida and Söderhäll, 1984; Söderhäll and Smith, 1986; Tsing et al., 1989; Sritunyalucksana and Söderhäll, 2000). Encapsulation has been observed in vas deferens and testicular intraspecific implants in *Macrobrachium rosenbergii* (Nagamine et al., 1980), as well as around stainless steel pins of disk tags introduced into *Penaeus (Farfantepenaeus) aztecus* (Fontaine and Lightner, 1973). Solid black capsules have also been produced around steel pins in the sub-genus *Litopenaeus* (Alfaro, unpublished data).

The intraspecific transplantation of androgenic glands in crustaceans has been studied in amphipods, isopods and decapods. The implantation of this organ into *M. rosenbergii* females induces masculinization, indicating that implanted tissues remain intact and active, metabolically and physiologically (Nagamine et al., 1980; Malecha et al., 1992). The aim of the present research was to study the recognition response of *P. vannamei* against thoracic nerve cord–ganglia transplants from *P. vannamei, P. stylirostris, Trachypenaeus byrdi* and *M. tenellum,* as a first step for defining tissue compatibility among shrimps, including three species of Penaeidae and one species of Palaemonidae.

2. Materials and methods

2.1. Recipient and donor species

Species used in this study were collected from Golfo de Nicoya, Pacific coast of Costa Rica. *P. stylirostris* (9–11 cm total length (t.l.)), *T. byrdi* (8–10 cm t.l.), and *M. tenellum* (7–8 cm t.l.) were captured from the wild, and kept in tanks at Estación de Biología Marina (EBM). *P. vannamei* were obtained from a commercial shrimp farm from the same gulf and grown to 13–15 cm t.l. at EBM in a shaded external tank (18 m^2). Water exchange was kept at 48% per day, using new water pretreated by high pressure silica sand filtration and sedimentation. Animals were fed a commercial dry food and fresh frozen sardine to satiation. P*enaeus* and *Trachypenaeus* belong to the family Penaeidae, and *Macrobrachium* to Palaemonidae.

2.2. Surgical procedure

The experiment was designed with *P. vannamei* as the only receptor species and four species as the tissue donors: a) control group = nerve tissue from *P. vannamei* , b) nerve tissue from *P. stylirostris,* c) nerve tissue from *T. byrdi,* and d) nerve tissue from *M. tenellum.* Donors from each species were kept alive until dissection. The entire thoracic nerve cord–ganglia chain was aseptically removed, sectioned in two-three pieces (5–6 mm in length each), and maintained in chilled (12–14 °C), sterile crustacean physiological solution (C.P.S.) with high HEPES concentration (160 mM, Talbot et al., 1989) for 5–10 min before implantation. A complementary control group was included using C.P.S. with low HEPES concentration (18 mM, Ro et al., 1990).

Each *P. vannamei* recipient received one tissue section, using an implantation device. The implanter was made with a syringe (1 ml) modified to carry replaceable sterile glass tubes (2 mm in diameter) to load tissue sections. Each recipient was implanted with a new glass tube to avoid contamination; alcohol was applied at the surface and a small hole was made with the tube through the soft epidermis at the dorsal junction between cephalothorax and abdomen, then the tissue was expelled into the cavity located at the left side from the heart. Recipients were maintained in indoor circular tanks (3 m in diameter) with pretreated water (temperature = 27 °C; salinity = 34 ppt) exchanged at 100% daily. Implants from every donor species were left in the recipients for 7 days, and additional periods (14, 27, 34 days) were tested in some species.

2.3. Implant analysis

Implanted tissues were removed from recipients at the end of the incubation period, catalogued based on three gross categories: a) missing, b) unmelanized, and c) melanized. Unmelanized and melanized tissues were further categorized based on their size: small (2–3 mm), and large (5–6 mm). Immediately, tissues were fixed in Davidson's solution for 24 h and transferred to ethanol 50% for storage (Bell and Lightner, 1988). Samples were dehydrated in ethanol, embedded in paraffin, sectioned (10 µm), and stained in hematoxylin plus eosin. Control non-implanted thoracic ganglia were also processed from three donors of each species by dissecting tissues and fixing as before. Cellular structure and integrity of transplants was compared to non-implanted tissues by histological analysis as applied by Nagamine et al. (1980), and tissue structure was based on the descriptions provided by Bell and Lightner (1988).

2.4. Absorption of grafts in P. stylirostris

A complementary experiment was performed to evaluate the absorption of intraspecific tissue sections in *P. stylirostris.* Thoracic nerve cord–ganglia sections (6 mm in length) were isolated in C.P.S. 18 mM HEPES and implanted in 9 recipients (13–14 cm in t.l.) as indicated previously. Three implants were removed at 7, 14 and 21 days for gross observations and length measurement.

3. Results

The surgical procedure developed for this research proved to be an efficient technique for delivering tissue sections into the shrimp body. Before adopting the dorsal cavity as the place of implantation, other regions were tested, such as the abdominal ventral surface and the abdominal dorsal surface. However, the dorsal cavity was chosen because the epidermis at this junction has no cuticle; therefore, tissue sections can be easily released into this region. Implants can be monitored with the naked eye, without disturbing recipient animals.

Post-surgical mortality was 0% and 22 % for recipients implanted with thoracic ganglia from *P. vannamei* using C.P.S.-18 mM and C.P.S.-160 mM, respectively. Recipients with thoracic ganglia from *P. stylirostris, T. byrdi,* and *M. tenellum* had mortalities of 21%, 53% and 10%, respectively (Table 1). Mortality rates from 0% to 22% are considered very low as compared to 89.5% reported by Malecha et al. (1992) for androgenic gland transplants in *M. rosenbergii.* High mortality was only observed in *P. vannamei* recipients implanted