Fishery Technology
2002, Vol. 39(2) pp : 94 - 99

Pattern of Pigment Migration in Second Cheliped of *Macrobrachium rosenbergii* with Morphotypic Transformation

K. Ranjeet and B. Madhusoodana Kurup
School of Industrial Fisheries
Cochin University of Science and Technology
Fine Arts Avenue, Cochin - 682 016, India

Changes in cell shape of chromatophores and subsequent pigment migration on the second cheliped of the three main male morphotypes of *Macrobrachium rosenbergii* are discussed. Cell enlargement as part of pigment accumulation in chromatophore was found to be maximum in Blue clawed males (1.5 µm), followed by Orange clawed males (1.1 µm) and Small males (0.65 µm) in male morphotypes of *M. rosenbergii*. The concentration of pigments at localized sites facilitated by chromatophoric extension through microtubules gave characteristic orange and blue colour to the cheliped, which could directly or indirectly be modulated by the chromtophoric hormones (CH) or pigment dispersing hormones (PDH).

Key words: *Macrobrachium rosenbergii*, Morphotypes, Chromatophores, Pigment Migration

Sexually mature single aged population of giant freshwater prawn, *Macrobrachium rosenbergii* is composed of three main morphotypes, named as Small Males (SM), Orange clawed (OC) males and Blue clawed (BC) males based on the colouration and appearance of the second cheliped. All the three morphotypes differ in their size, morphology, physiology and behaviour (Telecky, 1984; Ra'anan & Sagi, 1985; Kuris *et al.*, 1987; Harikrishnan & Kurup, 1997). These morphotypes represent different phases in the male developmental pathways (Ra'anan & Sagi, 1985). Each morphotype on the basis of its claw colour occupies a definite position in the social hierarchy either as dominant, subdominant of subordinate males (Cohen, 1981; Harikrishnan & Kurup, 1997).

It is well known that the crustacean chromatophore includes a stellate pigment-containing cell along with spatially fixed tubes through which pigment granules move either centrifugally or centripetally (McNamara, 1981a). The structural basis of pigment granule translocation within chromatophore has revealed the presence of well developed microtubular and microfibrillar filament systems within crustaceans (Robinson & Charlton, 1973). Although concerted attempts have been made to study the ultrastructure of various crustacean chromatophoric systems (McNamara, 1979; Bauer, 1981 and McNamara & Moreira, 1983), not much effort have been taken to bring out the mechanism of pigment migration and related structural changes during growth and maturation in crustaceans. In view of the paucity of information essential for the understanding of pigment translocation and cellular function, the present study was undertaken to examine the microanatomy of pigmentory effectors in *M. rosenbergii*.

Materials and Methods

Adult male specimens of freshwater prawn, *Macrobrachium rosenbergii* of the same age were collected from the farm at Kuttanad, South India and were transported in live condition to the laboratory. The prawns were segregated into three main morphotypes (Harikrishnan & Kurup, 1997) and were maintained in Oxford blue tanks for a couple of days. Live prawns were narcotized using 5% chloroform and 1 mm square pieces of exoskeletal tissue from the second cheliped were dissected out. These pieces were first fixed in 2.5% glutaraldehyde solution at 4°C for 18 h. Preparatory techniques for chromatosomes were followed as of McNamara (1981a). Later the samples were post fixed in 1% Osmium tetroxide solution for 2 h at 4°C. Tissues were then rinsed in 0.1 M phosphate buffer 2-3 times before dehydrating it in a series of acetone solution. Blocks for these tissues were prepared by embedding them in Araldite embedding medium. Ultra thin sections (0.25mm) on a transverse axis were cut using LKB Ultratome III and placed in thin Cu-Ni grids and stained with lead citrate before examining them under Philips C20 transmission electron microscope at an accelerating voltage of 60KV. All measurements were taken directly from the micrograph and were given as mean values. Significant difference between means ($p=0.05$) were tested using Duncan's t-test in SPSS 7.5 for Windows.

Results and Discussion

Figs, 1, 2 & 3 show exogenous vacuoles with pigment granules bordering it in the three main morphotypes of *M. rosenbergii* viz. SM, OC and BC respectively. Prominent electron dense spherical pigment granules were more in OC & BC than small males.

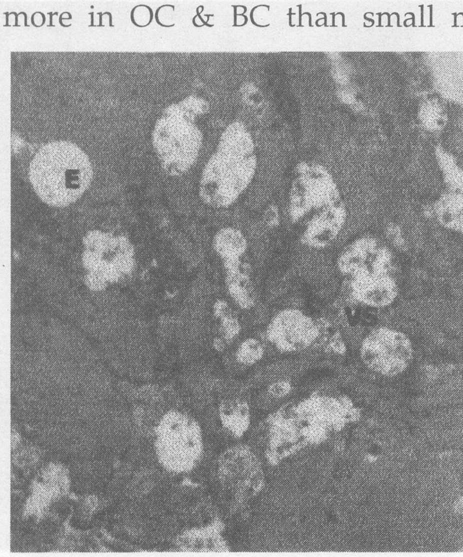

Fig. 1. Exogeneous vacuoles of SM

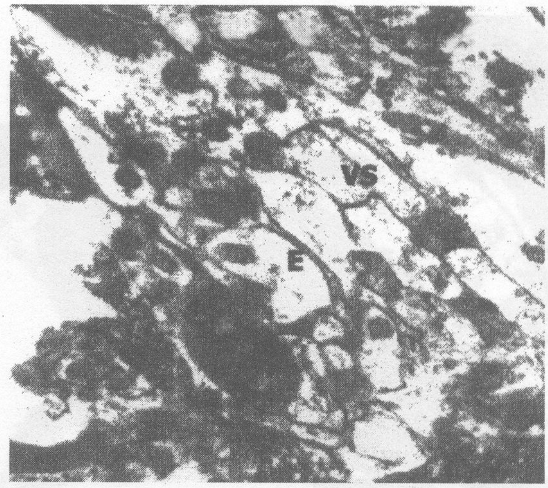

Fig. 2. Exogeneous vacuoles of OC

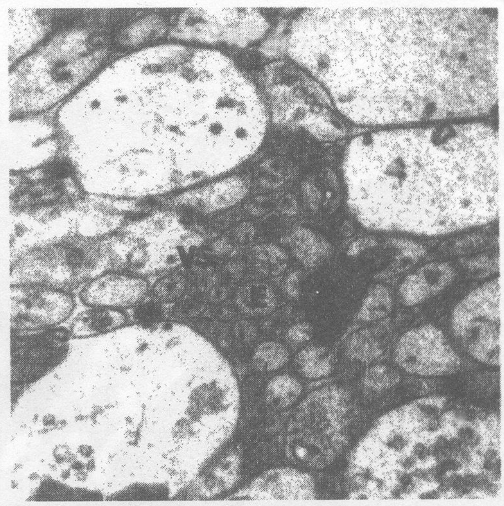

Fig. 3. Exogeneous vacuoles of BC

The size of these pigment granules was recorded to be 158±12 nm in BC followed by 96±16 nm in OC and 51±8 nm in SM. From the figure it was clearly visible that colour pattern of the claw of *M. rosenbergii* is formed by pigments within chromatosomes located beneath the cuticle of the second cheliped. The basic functional unit, the chromatophore, is a large asymmetrical cell consisting of roughly spherical cell body of approximately 15 μm diameter. With the transformation of SM to OC and further to BC, the shape and size of these chromatophore also change which technically is a process of cell extension. In SM cell extension was recorded to be 0.65 μm, while in OC and BC it was observed to be 1.1 μm and 1.5 μm respectively. Figs. 4, 5 & 6 show the general disposition of chromatophores

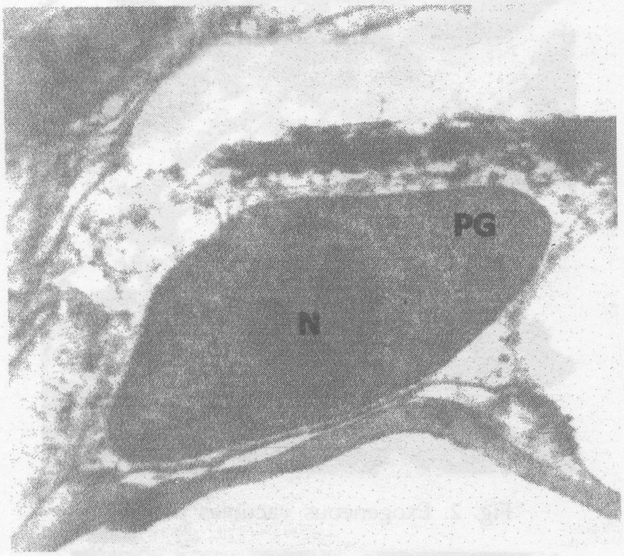

Fig. 4. Chromatosome of SM

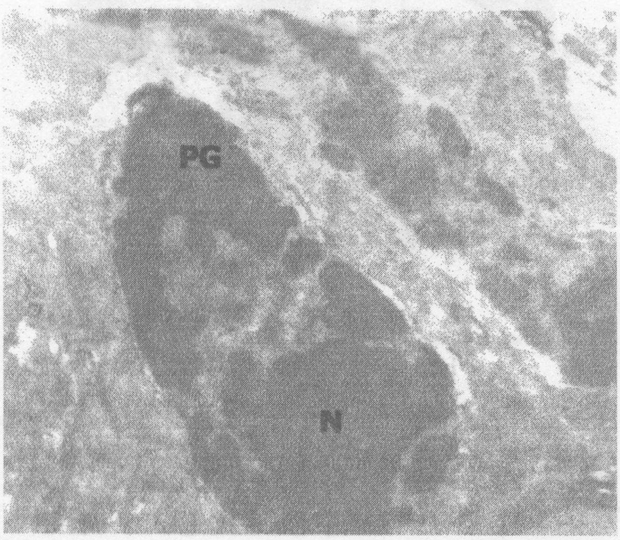

Fig. 5. Chromatosome of OC

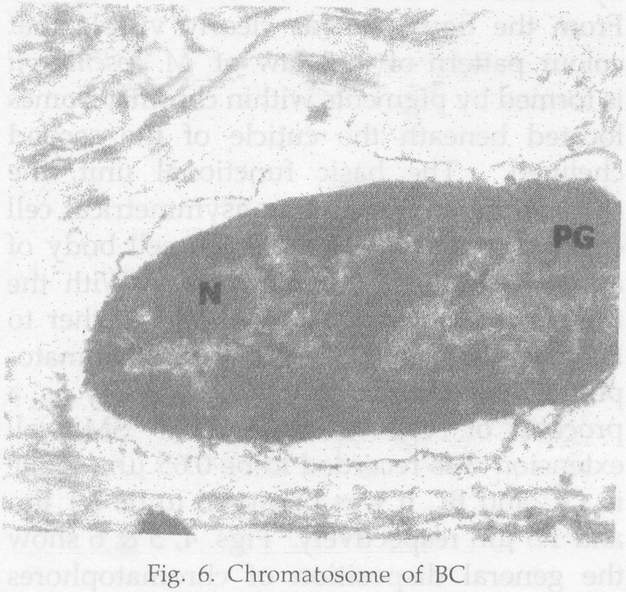

Fig. 6. Chromatosome of BC

with aggregated pigments comprising of chromatosomes in SM, OC and BC respectively. The pigments in all the three morphotypes had agglomerated to form a central mass within each chromatophore, while cell nucleus lies at the base of the cell extension. In the present study it was found that with more and more deposition of pigments taking place within the chromatophores, the nucleus gets shifted from its initial central position in SM to the base in OC and BC. In all the three morphotypes studied the cell body consisted of sparsely distributed vesicles of agranular endoplasmic reticulum and a few microtubules lining the chromatophores. It is by virtue of these microtubules that the pigments enter the chromatophore and in turn cause the cell extension in terminal morphotypes. Once the pigments are fully dispersed, the individuality of the chromatophore comprising the chromatosome becomes indistinct. In each chromatophore, the pigment now surrounds the nucleus that also appears to be displaced into the cell extension. The microtubule bundles do not appear to exhibit any preferential orientation within the cell body and no centers of microtubular organization are evident. Figs. 7, 8 & 9 shows the microtubular arrangement around the chromatophores in SM, OC & BC respectively. The microtubules of 36-42 nm external diameter run the entire

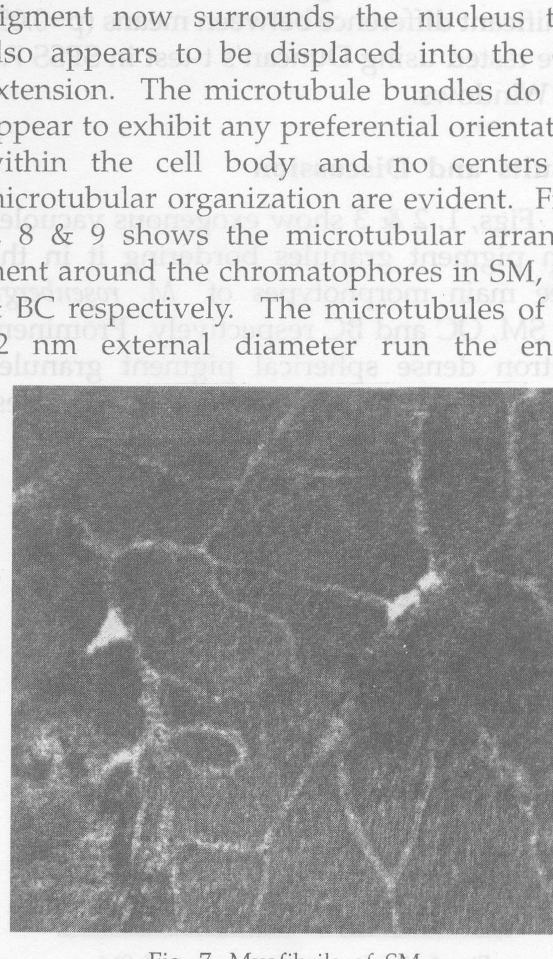

Fig. 7. Myofibrils of SM

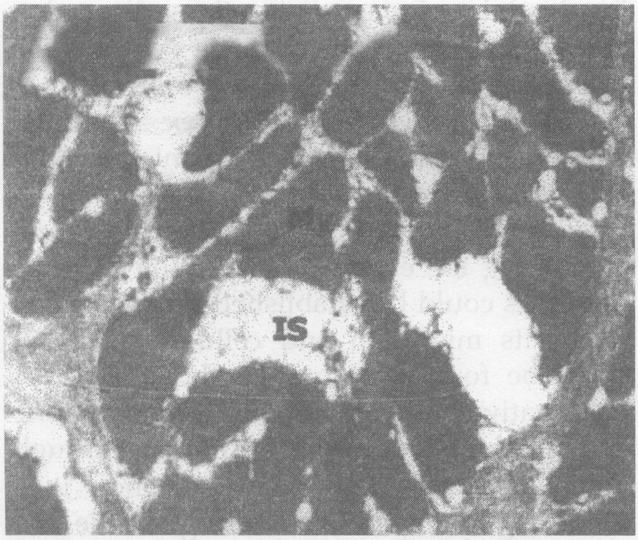

Fig. 8. Myofibrils of OC

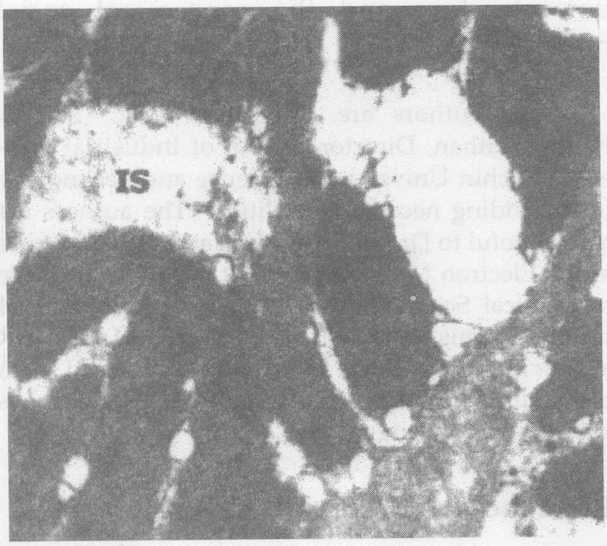

Fig. 9. Myofibrils of BC

length of the cell extension and typically lie parallel to the longitudinal axis of the projection of extension. The degree of cell extension is directly dependent on the spacing between the microtubules. In Fig. 7, the spacing is very less indicating only slight extension, but the spacing and in turn stretching of cells increases from OC to BC (Fig. 7 & 8). They present an even distribution throughout the cytoplasm exhibiting a centre to centre spacing of 78±8 nm and numerical density of 28±6 microtubules/mm^2.

The ultrastructural data presented in the current study defines the typical features of *Macrobrachium rosenbergii* chromatophore primarily in the second cheliped and demonstrates certain ultrastructural modifications associated with pigment movement. The chromatophoric pattern changes according to the growth of the prawn (Hiramatsu *et al.*, 1985) i.e. prawns of the same size have almost similar chromatophore pattern but the shade of colouration may differ in individuals. However, in *M. rosenbergii* a phenomenon of differential growth is evident among the single aged male population (Kuris *et al.*, 1987). The difference in somatic growth is complemented by a change in the colour pattern of the second cheliped also, which shifts from pale white in SM to dark orange in OC to peacock blue in BC. Along with the changes in other body characters the transformation of claw colour make the prawn attain subsequent morphotype status in its developmental pathway. It has been found that during transformation from SM to OC to BC, the muscle fibre type pattern remains the same, although there are marked changes in the size and colouration of the claws (Govind & Pearce, 1993). Although, earlier attempts were made to unravel the mechanism of colour change by studying the chromatophoric pattern in other palaemonids (McNamara, 1979), post larvae of *M rosenbergii* (Hiramatsu *et al.*, 1985) and embryos and adults of *M. olfersii* (McNamara, 1989 and McNamara & Taylor, 1987), the phenomenon of changing colour pattern of the claw with the transformation of morphototypes of *M. rosenbergii* has not yet been attempted so far. From the present study it is understood that in *M. rosenbergii* like any other decapod crustacean, the pigment cells are responsible for the colour shades of the second cheliped. Moreover, the intensity of pigment granules differs from morphotype to morphotype. The translocation of pigment granules are mediated by microtubules and the quality of their transfer to the cell may be modulated by chromatophoric or pigment dispersing hormones (PDH). A process of cell extension was also seen among morphotypes which points out that the chromatophore tends to bulge at specialized sites with the aid of microtubules to accommodate more and more pigments granules in it. This indiscriminate deposition of pigment granules and subsequent increase in the cell volume imparts shades of colour which gives the characteristics colour to a particular morphotype. Hence the intensity of colour, particular to each morphotype is determined by the quantity of pigment granules within the chromatophore than the type of pigment granules being accumulated.

During pigment aggregation, the entire volumes of the pigment and the cytoplasm appear to be transferred directly to the cell body. In general, the available literature suggests that microtubules provide the motive force for pigment granule translocation within chromatophores (Murphy & Tilney, 1974). McNamara & Taylor (1987) reported that microtubules from a central core surrounded by pigment granules within the cell extension of *Palaemon affinis* epidermal chromatophore contain dispersed pigments. The results obtained in the present study also agree with these observations. There appears to be a regular contraction or polymerization of the microtubules around the chromatophores. However, the possibility of depolarization is sparse, whereby the colour change in claws is progressing from pale white through orange to deep blue. The ultrastructural features of chromatophores with dispersed pigments seen in the present study are similar to that observed by McNamara & Taylor (1987) in *P. affinis* and *M. olfersii*. Once the pigments are fully dispersed the individuality of the chromatophore comprising the chromatosomes become indistinct. Qualitatively, the cytoplasmic contents of cell extension filled with pigments of OC & BC are similar to those of extension without pigments observed in SM.

Ultrastructural evidence from the current study indicates a complex phenomenon involved in pigment migration, dispersion and translocation mechanism within the chromatophore of three male morphotypes of *M. rosenbergii*. A complex structural relationship exists between the microtubular and chromatosome organelle systems. Although the production and external stimuli controlling the extent of dispersion of these pigments could be established in the present study, its migration and cellular extension could be found out only with the help of quantitative estimation of the chromatophoric and pigment dispersing hormones through Radio Immuno Assay (RIA). Hence, it is found highly imperative to bring out the role of various hormones on development of colour patterns characteristics of each morphotype of *M. rosenbergii* in the population structure and the consequent social hierarchy.

The authors are grateful to Prof. (Dr.) C. Hridayanathan, Director, School of Industrial Fisheries, Cochin University of Science and Technology for providing necessary facilities. The authors are also grateful to Dr. Taposh K. Das and his colleagues at the Electron Microscope lab at All India Institute of Medical Science, New Delhi for providing EM facilities. This work was done as part of an ICAR ad hoc project and the financial aid received from the Indian Council of Agricultural Research is greatly acknowledged.

References

Bauer, R.T. (1981) *Mar. Biol.* **64**, 141

Cohen, D., Ra'anan, Z. & Brody, T. (1981) *J. World Maricul. Soc.* **12**, 231 Govind, C.K. & Pearce, J. (993) *J. Crust. Biol.* **13**(1), 138

Harikrishnan, M. & Kurup, B.M. (1997) in *Proc. Ninth Kerala Sci. Cong.* January 1997, Trivandrum, Kerala, p.387

Hiramatsu, K., Uno, Y. & Horas, N. (1985) *Crustaceana*. **48**(2), 150

Kuris, M.A., Ra'anan, Z., Sagi, A. & Cohen, D. (1987) *J. Crust. Biol.* **7**, 219

McNamara, J.C. (1979) *J. Exp. Mar. Biol. Ecol.* **40**, 193

McNamara, J.C. (1980) *J. Exp. Mar. Biol. Ecol.* **46**, 219

McNamara, J.C. (1981a) *Biol. Bull.* **17**, 193

McNamara, J.C. (1989) *Crustaceana.* **571**, 38

McNamara, J.C. & Moreira, G.S. (1983) *Crustaceana*. **44**(3), 301

McNamara, J.C. & Taylor, H.H. (1987) *Crustaceana.* **53**, 113

Murphy, D.B. & Tilney, L.G. (1974) *J. Cell Biol.* **61**, 757

Ra'anan, Z. & Sagi, A. (1985) *Biol. Bull.* **169**, 592

Robinson, W.G. & Charlton, J.S. (1973) *J. Exp. Zool.* **186**, 279

Sagi, A. (1984) M.Sc. Thesis, Life Sciences Institute, Hebrew University of Jerusalem, Israel, pp.250

Telecky, J.M. (1984) *Pacific Science*. **38**, 372