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Future prospects of crustacean monosex culture: could giant prawn monosex culture benefit from the discovery of an insulin-like factor?

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In many crustacean species a sexual bimodal growth pattern is exhibited where females grow larger than males of the species or *vise versa*. In two of the most economically important penaeid shrimps, *Litopenaeus vannamei* and *Penaeus monodon*, females grow larger than males (Hartnoll, 1982). On the contrary, in several cultured species such as the Australian red-claw crayfish *Cherax quadricarinatus*, males grow faster and reach higher weights than females (Manor *et al.*, 2002). This is also the case for the giant freshwater prawn *Macrobrachium rosenbergii* (Sagi *et al.*, 1986), a species estimated to be cultured at over 200,000 tones annually (FAO, 2006). Since males of the latter species reach market size faster than the females, an all-male monosex population culture of the species is desirable.

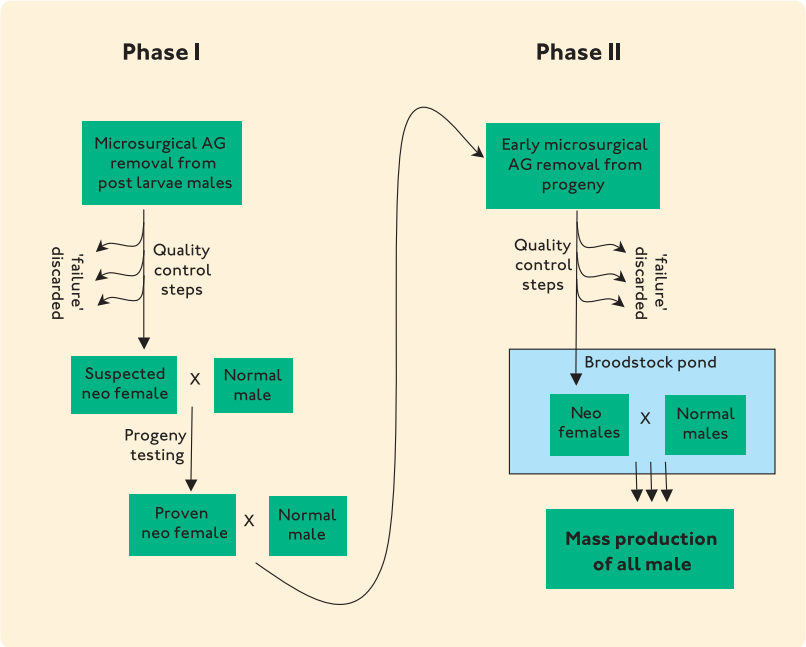

Figure 1: Schematic representation of a novel two-phase procedure for mass production of all-male *M. rosenbergii* populations. (source: Aflalo *et al.*, 2006).

Culture of monosex populations is a common procedure in animal husbandry. Differences between males and females of the same cultured species, in growth rate, alimentary needs and behavioral patterns, dictate the need to establish management procedures specifically adjusted to one sex or the other. Moreover, since a monosex culture population is inherently non-breeding, energy is diverted to growth and unwanted breeding is prevented. Reproduction can be carried out in such systems under separate, controlled conditions. The monosex culture strategy has become a common practice in fish culture and attempts have been made to apply it to crustacean culture. A small scale experiment was conducted by hand segregating *M. rosenbergii* monosex populations resulting with significantly higher yields when all-male populations were cultured (Sagi *et al.*, 1986). More recently, an economic analysis of all-male population culture showed income increase by ~60% over mixed and all-female populations, taking into account the expenses under Indian conditions, caused by labor-intensive hand segregation and related losses (Nair *et al.*, 2006).

A male specific endocrine gland which was shown to mediate male sexual differentiation in several crustacean species has drawn the attention of researchers interested in devising non-laborious techniques for monosex population culture in crustacean species exhibiting sexual bimodal growth patterns. This gland, known as the androgenic gland (AG), is separated from the gametogenic organs, the testes, enabling a targeted intervention in sex differentiation without tempering the gonads (Sagi *et al.*, 1997).

In *M. rosenbergii* a fully functional sex reversal was achieved by microsurgically dissecting out the AGs from early post-larval males. The neo-females (phenotypic females with male genotype) were crossed with males and gave rise to a 100% male progeny (Sagi and Cohen, 1990). However, the elaborated technique for AG ablation requires a highly skilled staff and the success rates, which are based on the identification of males at a very young stage, are low. A two phase scheme for the production of all-male population was devised where the progeny of successfully reversed males (100% males) served for microsurgical removal of the AG at an early stage without the need to identify males (Figure 1). This two phase procedure increased the success rates and, although involves a complicated microsurgical operation and time consuming progeny testing, it proved all male population production to be more feasible (Aflalo *et al.*, 2006). However, a more elegant and easy to use technique is still needed to enable the scale-up of monosex culture of the species throughout the world.

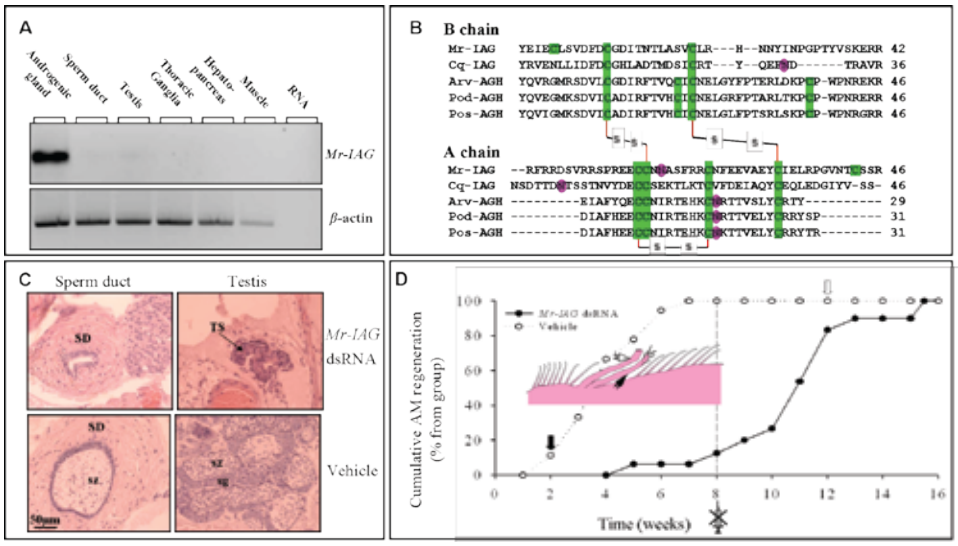

Figure 2: Identification and characterization of *M. rosenbergii* insulin-like AG (*Mr-IAG*) gene. (A) RT-PCR (reverse transcriptase-polymerase chain reaction) showing expression of *Mr-IAG* only in the AG. β-actin is used as positive control. RNA is used as negative control. (B) Multiple sequence alignment of the putative mature Mr-IAG peptide with all other known AG specific insulin-like peptides (Based on SMART results and done by ClustalW). Cysteine residues are marked by a green color. Conserved predicted disulfide bridges are linked. (C) Temporal silencing of *Mr-IAG* using dsRNA injections inhibits testicular spermatogenesis. In the silenced individual there is an empty sperm duct (SD) as oppose to the spermatozoa-filled sperm duct in the control individual. In the silenced individual there are inactive testis lobules (TS) as oppose to the active testis lobules in the control individual, containing both spermatogonia (sg) and spermatozoa (sz). (D) Temporal silencing of *Mr-IAG* using dsRNA injections inhibits regeneration of male secondary characteristic – the *appendix masculina* (AM). At the start of the injections all individuals had one AM removed. By the end of the repeated injections period (8th week), all of the vehicle injected individuals regenerated their AM as oppose to most of the silenced individuals, which did not. This inhibition in AM regeneration was reversible as all silenced individuals regenerated their AM by the 15th week.

The end of the repeated-injection period is marked as -  (8th week). In pink – an illustrated second pleopod with *appendix interna* (hollow arrowhead) and *appendix masculina* (black arrowhead). (Source: Ventura *et al.*, 2009).

Based on recent findings in a crayfish (Manor *et al.*, 2007), researchers tried to find specific AG genes responsible for male sexual differentiation in *M. rosenbergii*. A subtractive cDNA library of *M. rosenbergii*'s AG has been established from which, among other genes, an AG-specific gene, expressed exclusively in males was found. This gene was termed *Mr-IAG* (insulin-like AG factor from *M. rosenbergii*, Accession #FJ409645, Figure 2A) since its deduced protein sequence contains Cys residues and putative cleaved peptide patterns whose linear organization is similar to those of members of the insulin/insulin-like growth factor/relaxin family (Figure 2B). The function of this gene was elucidated via gene-silencing experiments which indicated that the gene affects spermatogenesis, the development of external male specific sex characters (such as *appendix masculina*) and also growth patterns (Figure 2C, D; Ventura *et al.*, 2009).

Since the gene was shown to be involved in growth, further research is required to harness this finding to the management of size variation in *M. rosenbergii*. We hypothesize that a complete functional sex reversal from males to neo-females might be achievable by manipulating genes such as *Mr-IAG* and other AG specific genes and proteins at earlier stages, thereby bearing the potential for the establishment of all-male populations in crustaceans. Such populations could be beneficial not only directly for yield increase in aquaculture, but also for restocking endangered and over-fished species, population control of invasive crustacean species as well as for sustainable aquaculture where it might reduce linkage of genetically selected lines into the natural ecosystem.

References:

Aflalo, E. D., T. T. T. Hoang, V. H. Nguyen, Q. Lam, D. M. Nguyen, Q. S. Trinh, S. Raviv, and A. Sagi. 2006. A novel two-step procedure for mass production of all-male populations of the giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture* **256**:468-478.

Hartnoll, R. G. 1982. *The Biology of Crustacea: Embryology, Morphology, and Genetics*. Academic Press, New York.

Manor, R., R. Segev, M. P. Leibovitz, and A. Sagi. 2002. Intensification of redclaw crayfish *Cherax quadricarinatus* culture II: Growout in a separate cell system. *Aquacultural Engineering* **26**:263-276.

Manor, R., S. Weil, S. Oren, L. Glazer, E. D. Aflalo, T. Ventura, V. Chalifa-Caspi, M. Lapidot, and A. Sagi. 2007. Insulin and gender: An insulin-like gene expressed exclusively in the androgenic gland of the male crayfish. *General and Comparative Endocrinology* **150**:326-336.

Sagi, A., and D. Cohen. 1990. Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquaculture* **21**:87-90.

Sagi, A., Z. Ra'anan, D. Cohen, and Y. Wax. 1986. Production of *Macrobrachium rosenbergii* in monosex population: yield characteristics under intensive monoculture conditions in cages. *Aquaculture* **51**:265-275.

Sagi, A., E. Snir, and I. Khalaila. 1997. Sexual differentiation in decapod crustaceans: role of the androgenic gland. *Invertebrate Reproduction and Development* **31**:55-61.

Ventura, T., Aflalo, E.D. and A. Sagi. 2009. Future prospects of crustacean monosex culture: could giant prawn monosex culture benefit from the discovery of an insulin-like factor?. *Endocrinology* **150**, in press.