GENETIC IMPROVEMENT OF PRAWNS

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SUMMARY

Progress in the genetic improvement of prawns has been slow because of a lack of knowledge on key aspects of their biology and because farming industries have been satisfied to access wild stocks for broodstock. The spread of diseases which threaten farmed prawn production worldwide has emphasised the need to develop fully domesticated stocks. However, data relevant to quantitative breeding work is only beginning to appear with heritability estimates for growth of 0.4-0.5, and responses to selection of 4%, and heritability estimates for survival to disease of 0.3-0.4 with response to seletion of 12%. Highly variable markers (RFLPs, RAPDs, AFLPs, mtDNA and microsatellites) have been developed that allow the genetic variation in prawn stocks to be assessed, and a start to be made to genome mapping in the rare situations where pedigreed stocks are available. Work has begun on the isolation and characterisation of genes that play an important role in growth, reproduction and disease resistance, and which might be of use in developing transgenic technologies.

Keywords: genetic improvement, prawns, *Penaeus*, heritability, genetic manipulation.

INTRODUCTION

The basic techniques to farm prawns were developed more than twenty years ago (AQUACOP 1983; Primavera 1985). Today, farm production is dominated by a few species: *Penaeus vannamei* in North and South America, *P. chinensis* in China, and *P. monodon* in Asia, with some production of *P. indicus*, *P. merguiensis*, *P. japonicus* and *Metapenaeus ensis* in Asia and *P. stylirostris* in the Americas and Pacific (Fast and Lester 1992). Prawn farming now provides some 25% of total prawn production worldwide.

The massive development of the industry has not been underpinned by sound technological developments in reproduction or disease control. In recent years, production in several parts of the world has been reduced by several different viral diseases (Lightner 1993), to the extent that prawn farming (worth about US$800 million) has effectively ceased in Taiwan, and is seriously threatened in Thailand (40% drop in an annual production worth US$2 billion) (Liao 1990; Primavera 1993). The only long-term solution to these problems is the development of disease-free or disease-resistent domesticated stocks (Wyban *et al.* 1993; Carr *et al.* 1996).

The aim of the present paper is to provide a general background for those not familiar with the area that illustrates the primitive state of affairs in prawn genetic improvement relative to established agriculture species, but one that offers an exiting area of research bringing wild species into domestication.

PRAWN BIOLOGY AND FARMING

Although there are variations among species, most penaeid prawns produce their young in one or two major spawning seasons. They broadcast their eggs which hatch within 24 hours into larvae which undergo marked morphological changes (usually 6 naupliar stages, 3 zoeal stages, and 2 mysis stages) during their two-week planktonic life. They first feed on phytoplankton after 3 days, and at 5 days include zooplankton, before assuming the body form of a shrimp and settling on the bottom, where they feed on benthic invertebrates. They achieve maturity in 6-12 months, and live for about 2 years. In culture, the females are eyestalk ablated to remove the source of a hormone that inhibits egg production. They are mated, usually by natural service, and the larvae are fed on algae, microencapsulated feeds and live *Artemia* (a small crustacean). Two weeks after settling, the young post larvae are shipped from the hatchery to ponds where they are reared to harvest size (5 months), fed on commercial pelleted feeds. Although animals have been reared to adulthood (12 months) and used to produce eggs, the spawning success of pond-reared animals is generally far less than that of wild-caught adults, and the industry continues to rely upon wild stocks. Fast and Lester (1992) provide an extensive review of marine prawn biology and farming methods.

The potential of genetic improvement of prawns has been recognised for some time (Primavera 1985; Malecha and Hedgecock 1989; Hedgecock and Malecha 1991), but there are few soundly based breeding programs (Wyban *et al.* 1993; Benzie 1994; Bedier *et al.* 1996). Recent advances in breeding and genetics have been reviewed extensively by Benzie (1997, 1998).

QUANTITATIVE GENETICS

The difficulty of producing large numbers of defined matings, and of providing adequately controlled rearing conditions has slowed efforts to estimate the heritability of quantitative traits of commercial importance. Success has now been achieved in rearing large numbers of families from defined matings for *P. vannamei* (Wyban *et al.* 1993), *P. monodon* (Benzie *et al.* 1997), and *P. japonicus* (Hetzel *et al.* 1997) and work is in progress with *P. stylirostris* (Bedier *et al.* 1996). This technical capacity will now provide the means to assess responses to selection and to define breeding programs to achieve the specific breeding goals.

Several estimates of heritability for growth are now available which, in general, give values of about 0.3-0.5 for full-sib data from all species tested, suggesting a good basis for selection for size (Table 1). Hetzel *et al.* (1997) reported realised heritabilities of about 0.2 for increased growth and 0.4 for lower growth in *P. japonicus*, but the response to selection for increased size in *P. vannamei* after one generation of selection was only 4.4% (Fjalestadt *et al.* 1997). There are large environmental effects on prawn growth and many of the estimates have large standard errors. Divergent responses to selection from those predicted should not be too surprising, given the difficulty of choosing animals with high breeding value from the limited data base available at present. This should improve as predigrees are built up, and information from several generations, and different relatives, becomes available. A *P. stylirostris* line

Table 1. Heritability estimates for commercially important traits in farmed prawns from the postlarval stage to harvest (fs is full-sib, hs is half-sib)

Species/Trait	Heritability	Design	Source
P. japonicus			
Weight at 185 days	0.27 ± 0.08	34 fs	Hetzel *et al.* 1997
Weight at 185 days	0.21* up	34 fs	"
Weight at 185 days	0.41* down	34 fs	"
P. monodon			
Total length at six weeks	0.08 ± 0.10	18S:36D, hs sire est.	Benzie *et al.* 1997
Total length at six weeks	0.59 ± 0.30	18S:36D, hs dam est.	"
Wet weight at six weeks	0.12 ± 0.02	18S:36D, hs sire est.	"
Wet weight at six weeks	0.56 ± 0.03	18S:36D, hs dam est.	"
Total length at ten weeks	0.12 ± 0.07	18S:36D, hs sire est.	"
Total length at ten weeks	0.30 ± 0.11	18S:36D, hs dam est.	"
Wet weight at ten weeks	0.10 ± 0.002	18S:36D, hs sire est.	"
Wet weight at ten weeks	0.39 ± 0.004	18S:36D, hs dam est.	"
P. stylirostris			
Total length postlarva 1	0.84 ± 0.79	6 fs	Lester 1988
Total length postlarva 1	1.02 ± 0.60	9 fs	"
P. vannamei			
Total length postlarva 1	0.33 ± 0.41	9 fs	Lester 1988
Total length postlarva 1	0.36 ± 0.40	10 fs	"
Total length postlarva 1	0.15 ± 0.32	9 fs	"
Total length postlarva	0.35 ± 0.17	10 fs	Fast and Lester 1992
Total length early juvenile	0.53 ± 0.27	8 fs	"
Total length early juvenile	0.60 ± 0.25	8 fs	"
Total length late juvenile	0.05 ± 0.06	9 fs	"
Weight at harvest	0.42 ± 0.05	34S:158D fs est.	Carr *et al.* 1996
Weight at harvest	0.45 ± 0.01	34S:158D fs est.	Fjalestad *et al.* 1997
Weight at harvest	0.50 ± 0.13	34S:158D hs dam est.	"
Survival to TSV challenge	0.22 ± 0.09	34S:158D hs dam est.	Fjalestad *et al.* 1997
Survival to TSV challenge	0.35 ± 0.03	34S:158D fs est.	"

* realised heritabilities from lines selected for higher (up) or slower (down) growth.

selected for high growth, however, produced smaller animals than the control line after one generation, and this was thought to have occurred as a result of the highly inbred nature of the stock (Bedier *et al.* 1996).

Stong environmental effects have been used by Lester (1988) to explain the high heritability in an experiment where *P. vannamei* had poor survival, and a low heritability where survival was good (Table 1). However, it is more likely that the strong effects of common rearing environment, and maternal effects, in experiments with small numbers of families, resulted in high experimental error. This explanation was used to account for the marked variability of heritability estimates of growth at early larval stages (not given here) where estimates exceeded 1.0 and errors extended over the entire range of heritability (0-1), (Lester 1988). The effect of common rearing environment and maternal effects is seen in the data for *P. monodon*, where the dam estimates, which include variation from these sources, is far greater than the sire estimates (Benzie *et al.* 1997). The reduction in heritability from 6week to 10 week old postlarvae led these authors to speculate whether this represented the reduction of maternal effects arising from nutriton derived from the egg, which would be stronger earlier in development. Data on the heritability of resistance to disease are rare, but estimates derived from maternal half-sib, and full-sib groups, of *P. vannamei* indicated significant heritability of survival to Taura Syndrome Virus (TSV) of about 0.2-0.3. The presence of some substantial genetic control was further supported by a 12% response to selection for increased survival of *P. vannamei* to TSV after one generation (Fjalestad *et al.* 1997). An inbred stock of *P. stylirostris* highly resistant to IHHNV has been developed (Bedier *et al.* 1996).

MOLECULAR VARIATION AND POPULATION STRUCTURE

Molecular variants have been used for some time on wild prawn populations in order to define different stocks for fisheries purposes. The lack of allozyme variantion observed in prawns meant it was not clear whether the lack of spatial variation in most studies reflected high dispersal throughout species ranges, or a lack of sensitivity of the technique (Hedgecock *et al.* 1982; Benzie 1998), although structure has been observed over scales of 1,000s of kilometres using allozymes (Benzie *et al.* 1992) and mitochondrial DNA (mtDNA) markers (Benzie *et al.* 1993). The close association of a decline in spawner productivity with reducing genetic variability of allozyme variants in *P. japonicus* farmed in Italy highlighted the possible deleterious effects of uncontrolled inbreeding (Sbordoni *et al.* 1987) and engendered attempts to maintain larger effective population sizes in breeding programs (Malecha and Hedgecock 1989).

Considerable variation in randomly amplified polymorphic DNA (RAPD) (Garcia *et al.* 1994), (mtDNA), short sequence repeat DNA (SSR or microsatellite DNA) (Garcia *et al.* 1996) and RAPDs (Garcia and Benzie 1995), demonstrated their potential for use as family specific markers. Variants at the COI mitochondrial gene in *P. vannamei* families were associated with individuals showing high growth, providing positive evidence that molecular variants could be used as markers for traits of commercial importance. Microsatellites isolated from penaeid prawns with great difficulty are characterised by large size (100 repeats or more), and have

degenerate ends making the design of effective primers difficult, and their application to mapping likely to be limited in the short-term (Moore *et al.* 1997). However, Amplified Fragment Length Polymorphisms (AFLPs) have proved highly variable and are being used to develop a linkage maps based on three generation pedigrees of *P. japonicus* (Moore *et al.* 1997) and *P. monodon* (Benzie *et al.* unpublished data).

GENOME STRUCTURE

Knowledge of the prawn genome structure is limited in most species to a basic karyotype of 2n = 86-92 composed of small, mostly metacentric or sub-metacentric chromosomes with no identifiable sex chromosome (see summary in Benzie 1998). Chow *et al.* (1990) estimated the genome size of four penaeid species by flow cytometry to be approximately 70% that of the human genome. The size of the mitochondrial genome falls in the range of 15-17kb in all species for which data exists (Bouchon *et al.* 1994). Crude restriction fragment length polymorphism (RFLP) maps of the mitochondrial genome (Bouchon *et al.* 1994) and gene sequence data from small sections of the COI and/or 16S ribosomal genes (Palumbi and Benzie 1991; Machado *et al.* 1993) showed a surprising degree of genetic difference between species (at least 9.4% genome divergence between subgenera). Intergeneric differences in shrimp (25%) were more divergent than between some orders of mammals (23%). Sequence data are otherwise restricted to the 18S ribosomal nuclear gene, three protein coding genes and two unkown genes (Benzie 1998).

The extent of genome divergence is the likely cause of difficulties in applying microsatellites isolated from one penaeid species to other penaeid taxa. The large number of chromosomes and the relatively large size of the genome and the considerable divergence between species of penaeids suggest that considerable effort will be required to develop the basic information of assistance to prawn genetic improvement such as genome mapping and physical mapping. The lack of permanent cell cultures in prawns (Bols 1991) will also slow physical mapping, disease characterisation and some transgenic approaches.

GENOME MANIPULATION (HYBRIDISATION, POLYPLOIDY, TRANSGENICS)

Male prawns produce a sperm in a chitinous package, a spermatophore, which they insert in the female who retains the spermatophore for several days to weeks, and fertilizes the eggs when they are released. Artificial insemination involves the manual extraction of the spermatophore and its insertion in the female. A hard membrane develops within 12-15 min of the eggs contacting seawater, making it difficult to obtain unfertilised eggs in vitro and placing constraints on in vitro fertilisation experiments, polyploidisation work and cryopreservation techniques (Clark and Griffin 1993). Cryopreservation of sperm has been achieved and further development of this technique will be of use in future breeding programs, but reports of the cryopreservation of eggs and early larval stages have not been substantiated.

The development of artificial insemination techniques has allowed several interspecies hybridizations: (female x male) *P. setiferus* x *P. stylirostris, P. setiferus x P. schmitti, P. monodon* x *P. penicillatus* and *P. penicillatus* x *P. monodon,* and *P. monodon* x *P. esculentus,*

but the survival to postlarvae was <1% in all cases and no maturation was observed in the few cases where hybrids were reared to adulthood (Benzie *et al.* 1995). Polyploid penaeids (triploids and tetraploids) have been successfully produced using chemical and temperature shock and the animals reared to early larval, and in one case, to late post larval stages (Xiang *et al.* 1992; Aquacop *et al.* 1993). Tetraploid *P. chinensis* reared to six months old demonstrated a growth rate 20% higher than the diploid controls (Xiang *et al.* 1992). The formation of haploid zygotes of *P. chinensis* (=*P. orientalis*) using irradiated sperm (Dai *et al.* 1993), and diploid gynogens in *P. orientalis* (Nan'er and Feng 1993), has been reported.

Vertebrate-like growth factor peptides have been detected in *P. vannamei*, *P. stylirostris* and *P. indicus*, and human growth hormone fed to *P. vannamei* larvae had a positive effect on growth of the larvae (Toullec *et al.* 1991) suggesting transgenic modifications using vertebrate material might be successful. DNA has successfully been introduced into prawn eggs by microinjection but transformation success has been poor (Cadoret *et al.* 1991; Gendreau *et al.* 1991; Preston *et al.* 1997). No report of the successful development of transgenic prawns has been published, and new delivery mechanisms will need to be developed for crustaceans.

DISCUSSION

It is clear that the long-term strategy required to address disease problems and improve production in the prawn farming industry involves the development of fully-closed life cycles, and of genetically improved strains. There are problems with the fecundity and egg quality of cultured stock that provide practical frustrations to the application of quantitative breeding approaches until they are overcome. For example, while the viral factors responsible for disease remain uncharacterised, the ability to develop effective challenges specifically targetting one disease, will not only delay the development of a resistent strain, but also an understanding of the basic mechanisms of response to disease by prawns.

The information available on prawn genetics is fragmented and primitive (eg even the nature of their sex determining mechanism is unknown). Basic technologies that would assist technical aspects of domestication are not available, or extremely limited in development (cell cultures, in vitro fertilization, polyploids, gynogens etc). What is known of their biology (eg rapid hardening of eggs in seawater, realtively large genome and the possession of large numbers of small chromosomes, lack of response of cells to vertebrate cell culture factors) suggests that the development of these basic tools will be challenging. However, the scenario provides a wealth of opportunity for researchers in areas as yet little explored.

Nevertheless, relatively small experimental programs have made headway over the last ten years in developing the basic husbandry approaches that have allowed a start to mapping genomes in three prawn species, and practical attempts to improve growth through selective breeding. Basic work on prawn endocrine control of reproduction is well underway, and other aspects of fundamental biology are receiving attention. The combined application of molecular tools and sound breeding programs will undoubtedly speed the full domestication of these

species at a rate far faster rate than many terrestrial species experienced, given adequate investment in the venture.

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REFERENCES

AQUACOP (1983) In "CRC Handbook of Mariculture", Vol. 1. pp. 105-121, editor J.P. McVey CRC Press, Miami, USA.

AQUACOP, Ledu, C. and Diter, A. (1993) *Aquaculture* **111**:315 (abstract).

Bedier, E., Ottogalli, L., Patrois, J., Weppe, M. and AQUACOP (1996) World Aquaculture '96. Book of Abstracts. World Aquaculture Society. pp. 32-33

Benzie, J.A.H. (1994) *Australasian Biotechnology*, **4**: 222-224.

Benzie, J.A.H. (1997) *Aquaculture*, (in press).

Benzie, J.A.H. (1998) *Aquaculture*, (in press).

Benzie, J.A.H., Ballment, E. and Frusher, S. (1993) *Aquaculture*, **111**:89-93.

Benzie, J.A.H., Frusher, S. and Ballment, E. (1992) *Aust. J. Mar. Freshwater Res.* **43**:715-725.

Benzie, J.A.H., Kenway, M., Ballment, E., Frusher, S. and Trott, L. (1995) *Aquaculture* **133**:103-111.

Benzie, J.A.H., Kenway, M. and Trott, L. (1997) *Aquaculture*, **152**:49-53.

Bols, N.C. (1991) *Biotech. Adv,* **9**:31-49.

Bouchon, D., Souty-Grosset, C. and Raimond, R. (1994) *Aquaculture*, **127**:131-144.

Cadoret, J.P., Mialhe, E., Gendreau, S., Ohresser, M. and Le Deuff, R. M. (1991) ICES Mariculture Communication. ICES 1991 F:21. 5 pp.

Carr, W.H., Fjalestad, K.T., Godin, D. Swingle, J., Sweeney, J.N. and Gjedrem, T. (1996) World Aquaculture '96. Book of Abstracts. World Aquaculture Society. p. 63.

Chow, S., Dougherty, W.J. and Sandifer, P.A. (1990) *J. Crust. Biol.* **10**:29-36.

Clark, W.H. and Griffin, F.J. (1993) In "CRC Handbook of Mariculture", Vol I. pp. 133-151, editor J.P. McVey, CRC Press, Boca-Raton, USA.

Dai, J., Bao, Z., Zhang, Q. and Liu, J. (1993) *J. Ocean. Univ. Qingdao* **23**:151-155.

Garcia, D.K., Dhar, A.K. and Alcivar-Warren, A. (1996) *Mol. Mar. Biol. Biotech.* **5**:71-83.

Garcia, D.K., Faggart, M.A., Rhoades, L., Alcivar-Warren, A., Wyban, J., Carr, W.H., Sweeney, J.N. and Ebert, K.M. (1994) *Mol. Mar. Biol. Biotech.* **3**:270-280.

Garcia, D.K. and Benzie, J.A.H. (1995) *Aquaculture*, **130**:137-144.

Gendreau, S., Delecheneau, J.M., Cadoret, J.P. and Mialhe, E. (1991) European Aquaculture Society Special Publication No 14. pp. 115-116 (abstract).

Fast, A.W. and Lester, L.J. (1992) "Marine shrimp culture: principles and practices". Elsevier, Amsterdam.

Fjalestad, K.T., Carr, W.H., Lotz, J., Sweeney, J.N. and Gjedrem, T. (1997) Sixth genetics in Aquaculture Symposium Conference abstracts, Stirling, Scotland.

Hedgecock, D. and Malecha, S. R. (1991) In "Shrimp culture in North America and the Caribbean", *Advances in World Aquaculture* Vol 4. pp. 161-200, editor P.A. Sandifer, World Aquaculture Society, Baton Rouge, Louisiana, USA.

Hedgecock, D., Tracey, M.L. and Nelson, K. (1982) In "The biology of Crustacea", pp. 284-403, editor L.G. Abele, Academic Press, New York, USA.

Hetzel, D.J.S., Crocos, P.J., Davis, G.P., Moore, S.S. and Preston, N. (1997) Sixth genetics in Aquaculture Symposium Conference abstracts, Stirling, Scotland.

Lester, L.J. (1988) *Aqua. Fish. Mgnt.* **19**:243-251.

Liao, I.C. (1990) In "The Second Asian Fisheries Forum", pp. 11-27, editors Hirano, R. and Hanyu, I., Asian Fisheries Society, Manila, Philippines.

Lightner, D.V. (1993) In "CRC Handbook of Mariculture" Vol I. pp. 393-486, editor J.P. McVey, CRC Press, Boca-Raton, USA.

Machado, E.G., Dennebouy, N., Suarez, M.O., Mounolou, J.-C. and Monnerot, M. (1993) *Crustaceana* **65**:279-286.

Malecha, S.R. and Hedgecock, D. (1989) Sea Grant Technical Report UNIHI-SEAGRANT-TR-89-01. University of Hawaii Sea Grant College Program, Honolulu, USA. 40 pp.

Moore, S.S., Whan, V., Davis, G., Byrne K., and Preston, N. (1997) Sixth genetics in Aquaculture Symposium Conference abstracts, Stirling, Scotland.

Nan'er, C. and Feng, L. (1993) *Aquaculture* **111**:314 (abstract).

Palumbi, S.R. and Benzie, J.A.H. (1991) *Mol. Mar. Biol. Biotech.* **1**:27-34.

Preston, N., Leopold, R., Henderling, J. Atkinson, P.W. and Baile, V.J. (1997) Sixth genetics in Aquaculture Symposium Conference abstracts, Stirling, Scotland.

Primavera, J.H. (1985) In "Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps" pp. 47-64, editors Y. Taki, J.H. Primavera and J.A. Lobrera, Iloilo City, Philippines.

Primavera, J.H. (1993) *Rev. Fish. Sci.* **1**:151-201.

Sbordoni, V., La Rosa, G., Mattoccia, M., Cobolli-Sbordoni, M. and De Matthaeis, E. (1987) In "Selection, Hybridization and Genetic Engineering in Aquaculture", pp. 143-155, editor K. Tiews, Heenemann Verlag, Berlin.

Toullec, J.-V., Le Moullac, G., Cuzon, G. and Van Wormhoudt, A. (1991) *Aquat. Living Res.* **4**:125-132.

Wyban, J.A., Swingle, J.S., Sweeney, J.N. and Pruder, G.D. (1993) *World Aquaculture* **24**:39-45.

Xiang, J.H., Zhou, L.H., Liu, R.Y., Zhu, J.Z., Li, F.H., Li, X.H. and Liu, X.D. (1992) Proceedings of the Asia Pacific Conference on Agricultural Biotechnology, Beijing. China Science and Technology Press, Beijing. pp. 841-846.