

Integration of quantitative and molecular genetics in shrimp breeding

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Abstract

The increasing quantity of high quality DNA sequence, and proteomic, data is providing more efficient means of selecting strains and understanding physiology. Whole genome selection and the demonstration that high density single nucleotide polymorphism (SNP) analysis provides more accurate pedigree information in dairy cattle than the paper pedigree trail has revealed the prospective strengths of these approaches. In principle applicable to shrimp, fundamental differences between shrimp and vertebrate biology means the level of information available for shrimp is far less than that for cattle, pigs, chickens or finfish. Pedigreed data is far less in spatial and temporal extent and covers a relatively limited number of traits. Molecular markers allow parentage tracking and assessment of diversity levels in breeding programs. Work is in progress on the molecular and genetic mechanisms controlling key aspects of performance, including growth, reproduction and disease response. The success of the few attempts to integrate available molecular tools is limited by the lack of depth of information on shrimp and a lack of investment in the process. More effort will be required to obtain the critical research mass and quality of information needed to achieve true integration of molecular and quantitative genetics in shrimp breeding.

Introduction

The increasing quantity of high quality DNA sequence data, and proteomic data, is leading to ever greater amounts of molecular information on a variety of organisms. Most information is available for humans, in which considerable investment has been made, but agricultural species, both plants and animals have increasing amounts of molecular information (e.g. Collins et al. 2003; Varshney et al. 2009). However, investment levels in agriculture are an order of magnitude less than for humans, and this investment has been spread over several species. Nevertheless, molecular approaches are playing a leading role in understanding physiological

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processes, and providing molecular markers to assist selective breeding (Guimarães et al. 2007). The latter includes selection on traits that could not be selected previously because the phenotypes could not be distinguished by observation, and traits for which the molecular information increases the efficiency of selection. The ability to use whole genome selection and the demonstration that high density SNP analysis provides more accurate pedigree information in cattle than the paper pedigree trail, has revealed the potential power of the new approaches to genetic improvement in agriculture.

In principle, these approaches are equally applicable to aquaculture (Davis and Hetzel 2000). However, the level of investment in molecular genetics work in aquaculture species is an order of magnitude less than that for agriculture species. These issues are particularly acute for shrimp. Molecular markers are available that allow parentage tracking and assessment of diversity levels in breeding programs. There is work in progress on the molecular and genetic mechanisms controlling key aspects of performance, such as growth, reproduction and disease response. It is clear that integrating these approaches will benefit the shrimp industry, but the extent to which developments in quantitative and molecular genetics are integrated in shrimp aquaculture is not obvious.

This paper provides a brief review of the history of shrimp farming, the available information on shrimp quantitative and molecular genetics and assesses the extent to which these approaches have been integrated in shrimp genetic improvement. The scientific names used in this paper follow the recommendations of Alderman et al. (2007), using *Penaeus* as the preferred generic name for the common farmed species, but noting their sub-generic name (elevated by some authors to generic level) at first usage. All species discussed here belong to the family Penaeidae.

Brief History of Shrimp Farming and Shrimp Breeding Programs

The domestication and genetic improvement of penaeid shrimp has been reviewed recently (Benzie 2009). More details can be obtained from that source, but the main points are summarized here. Although some form of rearing shrimp through wild capture of larvae has been performed for many hundreds of years, the earliest development of large scale farming was in Japan in the 1950's and 1960's (Fast and Lester 1992; Liao and Chien 1994; Rosenberry 2002). This innovation took some time after the key technology development in the 1930's and 1940's, when rearing techniques for the kuruma prawn, *Penaeus (Marsupenaeus) japonicus* were developed in Japan by Hudinaga (1935, 1942). The development of large scale hatchery processes and better understanding of the rearing of shrimp in farms was required first (Liao and

Chien 1994). Since then, hatchery methods for more than 20 penaeid species have been developed and farm trials have been undertaken for many of these (Fast and Lester, 1992; Rosenberry, 2002; Briggs et al. 2004) (Table 1). Although a number of these species continue to be farmed locally, few have played a significant role in aquaculture production. Today, seven penaeid species, *Penaeus (Fenneropenaeus) chinensis*, *Penaeus (Fenneropenaeus) indicus*, *Penaeus (Fenneropenaeus) merguensis*, *Penaeus (Litopenaeus) stylirostris*, *Penaeus (Litopenaeus) vannamei*, *Penaeus (Marsupenaeus) japonicus* and *Penaeus monodon*, provide 99% of the world's farmed marine shrimp and two of these, *P. vannamei* and *P. monodon*, contribute 87% (Table 1).

Table 1. Species of penaeid shrimp for which hatchery technologies were developed and/or farm trials were carried out in the last 40-50 years, and the percent of world production for 2006, the latest available statistics (FAO 2009), for the main farmed species today.

Species for which hatchery technologies and/or farm trials were carried out	Principal species farmed today	% of market
<i>Metapenaeus affinis</i>	<i>Penaeus (Fenneropenaeus) chinensis</i>	2
<i>Metapenaeus ensis</i>	<i>Penaeus (Fenneropenaeus) indicus</i>	1
<i>Metapenaeus monoceros</i>	<i>Penaeus (Marsupenaeus) japonicus</i>	2
	<i>Penaeus (Fenneropenaeus) merguensis</i>	3
<i>Penaeus (Farfantepenaeus) aztecus</i>	<i>Penaeus monodon</i>	25
<i>Penaeus (Farfantepenaeus) brasiliensis</i>	<i>Penaeus (Litopenaeus) stylirostris</i>	<1
<i>Penaeus (Farfantepenaeus) californiensis</i>	<i>Penaeus (Litopenaeus) vannamei</i>	62
<i>Penaeus (Farfantepenaeus) duorarum</i>		
<i>Penaeus esculentus</i>	(all other species)	<5
<i>Penaeus (Farfantepenaeus) notialis</i>		
<i>Penaeus (Litopenaeus) occidentalis</i>		
<i>Penaeus (Farfantepenaeus) paulensis</i>		
<i>Penaeus penicillatus</i>		
<i>Penaeus plebejus</i>		
<i>Penaeus (Litopenaeus) schmitti</i>		
<i>Penaeus semisulcatus</i>		
<i>Penaeus (Litopenaeus) setiferus</i>		
<i>Penaeus (Farfantepenaeus) subtilis</i>		

The relative production levels of all of these species have fluctuated considerably through time (Fig. 1). *Penaeus japonicus*, the first species farmed, dominated for some 15-20 years, but now only supplies 2% of the world market; it required an expensive high protein diet and was displaced by shrimp that could be produced more cheaply. Other species became dominant depending on the status of disease, a major influence on shrimp farming (Walker and Mohan, 2009). A domesticated stock of *P. stylirostris*, resistant to infectious hypodermal and hematopoietic necrosis virus (IHHNV) was introduced to Central and South America

and to some Asian countries in the late 1990's under the name of "supershrimp" (SEAFDEC, 2005).

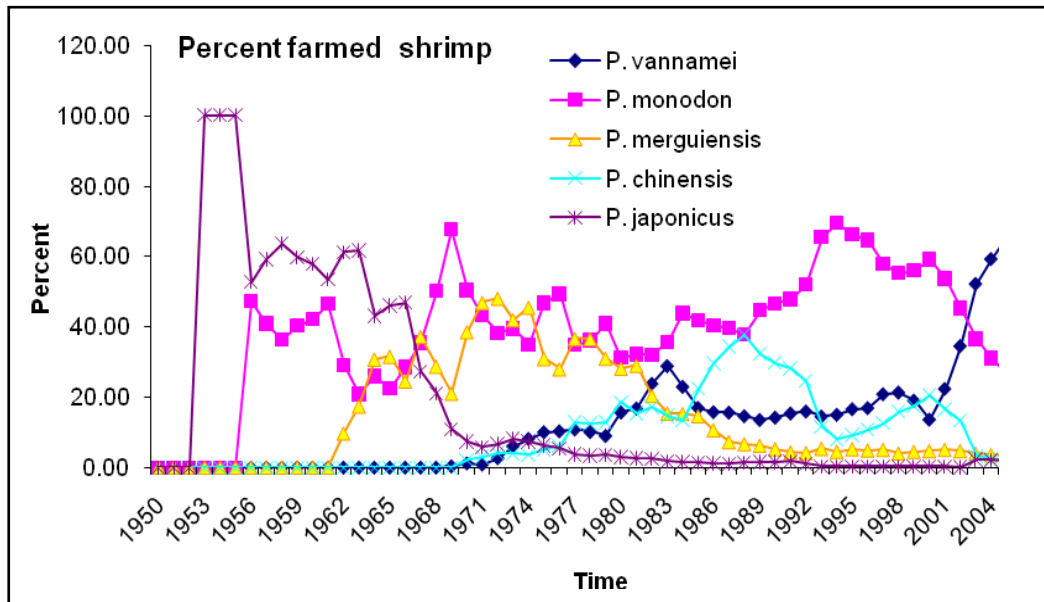


Fig. 1. Graph demonstrating the changing proportion of different species in the world production of shrimp, based on published FAO data (FAO 2009). A graph of total shrimp production is provided below for comparison. The category for unidentified farmed shrimp was excluded in calculating the proportion of production attributable to particular species (this effectively assumes that the proportion of particular species of shrimp in the unknown category reflects their proportions otherwise in the world market). The value of unidentified shrimp is less than 10% (usually less than 5%) for most of the time period so the figures would be affected little, if at all, should that assumption be violated. The figure for unidentified shrimp is much higher in earlier years 1950's and 1960's, but in this period *P. japonicus* and *P. monodon* were the only farmed species of note. *Penaeus japonicus* was dominant in the 1950's and 1960's, declining to less than 2% by the late 1970's; *P. merguensis* provided around 30% of world production in the mid-1970's declining to less than 5% by the late 1980's; *P. chinensis* rose in the late 1980's to about 35% declining to less than 3% by 2004. *Penaeus monodon* first appeared in significant volume in the late 1950's, maintaining a dominant market share until the late 1990's when the introduction of domesticated SPF strains of *P. vannamei* to Asia, led to a huge increase in production of this species. Comparison with the graph for total production (Fig. 2) shows that the volumes of *P. japonicus* and *P. merguensis* production, although dominant in the early years was never high compared with the large volumes that developed since the mid 1980's.

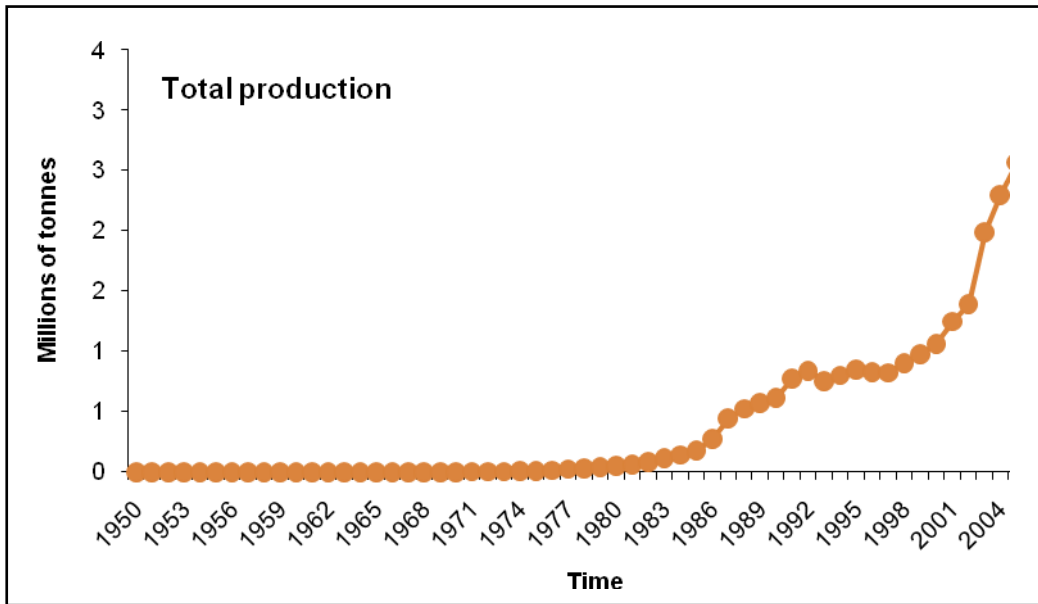


Fig. 2. Graph showing the total world production of shrimps from 1950-2004.

The stock proved susceptible to other diseases such as Taura syndrome virus (TSV) and white spot syndrome virus (WSSV), preventing this species from developing a dominant role in world production, despite being one of the first species for which domesticated strains were developed (Bedier et al. 1998). In contrast, a domesticated strain of *P. vannamei* resistant to TSV meant production of this species increased. The difficulty of developing domesticated stocks of *P. monodon*, a species with excellent farming traits now hampered by poor performance as a result of disease and lack of specific pathogen free (SPF) stocks, has seen this species' dominance of the market replaced by *P. vannamei*. History has therefore seen a succession of dominance in global supply by *P. japonicus*, *P. monodon* and *P. vannamei* respectively (Fig. 1). The relative production of other species through time depended on the geographic regions being brought into aquaculture production as a response to disease elsewhere and other market variable.

The history of breeding programs for shrimp is relatively short, with the first attempts to maintain domesticated populations over a number of generations being documented in the late 1980's (Benzie 1998, 2000). Given the widely known benefits of genetic improvement in agriculture, one might have thought that carefully planned approaches to breeding in aquaculture would have been used to achieve a major and rapid improvement in production. However, the actual history of development was one in which the key factors driving domestication were reliability of supply (which could not be achieved by accessing seasonal wild stocks) and the maintenance of production

in the face of disease through the development of disease tolerant or disease free stocks (Benzie, 2009). The development of domesticated strains of shrimp was therefore a response to critical issues affecting production, rather than a proactively built strategy for genetic improvement.

The animals used to make up the founding populations were usually those obtained from stocks available to the farmer/industry, including those derived from other farms and those which may have been under culture for a various number of generations (Benzie 2009). The founding populations were not established with particular forethought as to the genetic diversity present in the population, or with identified strategies for genetic improvement in mind. It is not a surprise that early genetic work highlighted concerns over the deleterious effects of unintended inbreeding through poor stock management (Sbordoni et al. 1986; Sbordoni et al. 1987). Domesticated stocks available to the industry were developed for their general production characteristics and were not designed to capture a particular subset of natural variation. As a result, a number of major domestication programs, which later morphed into genetic improvement programs, introduced new stocks to increase their genetic diversity, such as the U.S. Shrimp Consortium Program, which focused on *P. vannamei* (Alcivar-Warren et al. 2009) and the New Caledonia program for *P. stylirostris* (Goyard et al. 2003, 2008). More recent programs for *P. monodon* and *P. vannamei* have included a wide range of genetic diversity given the experience of the earlier programs (Gitterle et al. 2005a, 2005b; Argue et al. 2008) and larger programs have implemented improved stock handling techniques to prevent breeding of close relatives. The general history of the major domesticated strains has been documented in a range of published information summarized in Benzie (2009). It is clear that the genetic diversity in domesticated stocks depends on the original variation in the founder stocks and the nature of the regime used to manage the population. Most of the few existing major programs appear to have robust genetic and biosecurity management regimes (Lotz et al. 1995; Lotz 1997; Moss and Argue 2001; Le Moullac et al. 2003).

Domesticated stocks exist today for all of the main farmed species, often for private use of the company or industry owning the stocks, but not all are subject to genetic improvement programs (see Benzie 2009). Nevertheless, SPF genetically improved stocks are now available and openly traded for the two most commonly farmed species *P. vannamei* and *P. monodon* and almost all of the small *P. stylirostris* industry relies upon a domesticated stock. Domesticated stocks are playing an increasing role as a source of seed to farms with more than 99% of *P. vannamei* stocks being supplied from improved strains (Benzie 2009). Therefore, in total, about 70% of farmed shrimp are derived from domesticated stocks now, compared with the 1-2% estimated by Gjedrem (2000) a decade ago. Strains of SPF *P. vannamei*, improved for

growth and disease resistance in the U.S. Shrimp Consortium Program, have been spread worldwide and comprise all or parts of various breeding populations around the world. These populations and two strains developed separately by a U.S. Company and by an industry-wide program in Colombia have improved growth performance and comprise the main source of *P. vannamei* seed. An industry-wide program in New Caledonia has developed improved *P. stylirostris* and supplies a small, but important regional industry in the Pacific (Goyard et al. 2003, 2008). An SPF population of *P. monodon* improved for growth has recently been developed by the private sector in the U.S.A. (Argue et al. 2008), and breeding populations of *P. monodon* with improved reproductive performance, growth rates and farm yields have been established in Australia (Preston et al. 2009). Selectively improved growth of *P. chinensis* in China has been reported for a government-industry supported breeding program (Zhang et al. 2005; Huang et al. 2008), but the extent to which this strain supplies the industrial market is unknown.

The major point emerging from this brief review of shrimp farming is that the industry has few defined genetic improvement programs, although domestication is widespread. The number of species farmed has reduced greatly, with nearly all production now derived from seven species and most from only two. Production is increasingly dependent upon domesticated stocks and future production will demand more sophisticated approaches to genetic improvement of these populations.

Quantitative Genetic Information

Data on quantitative genetic parameters has been published for only a subset of the seven species most commonly farmed today. There are estimates for heritability of a variety of characters for *P. chinensis* (Huang et al. 2008; Zhang et al. 2008), *P. japonicus* (Hetzl et al. 2000), *P. monodon* (Benzie et al. 1997; Benzie 1998; Jarayabhand et al. 1998; Kenway et al. 2006; Macbeth et al. 2007), *P. stylirostris* (Bedier et al. 1998; Goyard et al. 2002) and *P. vannamei* (Carr et al. 1997; Fjalestad et al. 1997; Pérez-Rostro et al. 1999; Argue et al. 2002; Pérez-Rostro and Ibarra 2003a, 2003b; Arcos et al. 2004, 2005; Ibarra et al. 2005, 2007a; 2009; Gitterle et al. 2005a, 2005b, 2006a, 2006b, 2006c; Castillo-Juarez et al. 2007; Ibarra and Famula 2008). Fewer report realized heritabilities or responses to selection (Hetzl et al. 2000; Argue et al. 2002; Goyard et al. 2002; Preston et al. 2004; De Donato et al. 2005; Li et al. 2005), genetic correlations (Pérez -Rostro et al. 1999; Pérez -Rostro and Ibarra 2003a, 2003b; Arcos et al. 2004, 2005; Kenway et al. 2006; Macbeth et al. 2007; Ibarra et al. 2009) or genotype by environment interactions (Argue et al. 2002; Jerry et al. 2006b; Ibarra and Famula 2008). All these genetic parameters provide valuable information upon which to design genetic improvement programs. In a review of genetics in shrimp

breeding written more than a decade ago, Benzie (1998) reported fewer than ten papers giving quantitative genetic data for shrimp. Since then, there has been an increase in the number and accuracy of estimates of heritability and for a greater range of characters. However, progress over the decade has been less than might have been expected, and there are still fewer than forty original papers publishing detailed quantitative genetic information for penaeid shrimp (Benzie 2009). The majority of published data refers to *P. monodon* and *P. vannamei* (Table 2).

Heritability provides an assessment of the extent to which characters are under additive genetic control (i.e. due to allelic effects passed on from parents to progeny in gametes) and can be selected in the test populations, with the upper limit of 1.0 indicating that all of the variability in a population observed for the trait is due to allelic effects (i.e. complete additive genetic control) and the lower limit of 0 indicating no additive genetic control (all observed variation is then due to non-additive genetic effects e.g. dominance, epistatic or environmental effects). There would be no prospect of genetic improvement through selection for characters with heritabilities of 0. In general, characters with heritabilities less than 0.1 are unlikely candidates for economically viable selection, but those with values of 0.2 or above would be likely to show a good response to selection. Estimates for heritability have focused principally on various measures of growth (weight, length and size increments) and data exists for four species, with most estimates clustering around 0.3-0.5 (Table 2), indicating considerable scope for selection to be effective. Realized heritabilities and responses to selection confirm these levels of heritability in growth related characters with positive responses to selection for growth in *P. chinensis* (Li et al. 2005), *P. japonicus* (Hetzl et al. 2000; Preston et al. 2004), *P. stylirostris* (Goyard et al. 2002) and *P. vannamei* (Argue et al. 2002; De Donato et al. 2005).

Similarly, heritabilities of 0.14-0.62 have been measured for TSV resistance (Table 2) and positive responses to selection have been observed, with reductions in viral titre suggesting disease resistance rather than disease tolerance (Srisuvan et al. 2006). In contrast, heritabilities for resistance or tolerance to WSSV have been very low (<0.1 to 0), suggesting genetic selection is not possible, or will be very expensive, for that trait (Gitterle et al. 2005b, 2006a, 2006b, 2006c).

In *P. monodon* and *P. vannamei*, a number of reproductive traits such as number of spawns, days to spawn, egg number, nauplii number, hatch rate and biochemical characteristics of hemolymph (vitellogenin), of the eggs (protein, acylglyceride and vitellin levels) and stress related traits such as survival under hypoxia, have been measured and have high heritabilities (Table 2). Interestingly, egg size itself did not have a high heritability but oocyte size does (Arcos et al. 2005; Ibarra

et al. 2009). Genetic correlations between these reproduction related characters and between them, and weight or growth measurements were variable (see Ibarra et al. 2007b for a review of reproductive work), but those between oocyte diameters and hemolymph vitellogenin correlation was high (Ibarra et al. 2009). These data indicate the potential to improve a number of reproduction related characters through selection.

Genetic correlations between various weight and length measurements are generally high and positive (range $-.20$ to $+1.00$, majority >0.80), indicating that selection of any one of a number of measurements of growth would be effective. Positive or non-significant correlations of growth with TSV resistance are also reported (Carr et al. 1997). In contrast, the genetic correlations measured between growth and WSSV resistance ($-.94$ to $+0.33$, mean -0.24) are largely negative (Gitterle 2005b, 2006c). These results, together with data for low heritability of WSSV resistance, indicate selection for WSSV resistance would select for slow growth and vice versa. This suggests the development of WSSV tolerant strains are likely to be uneconomic and other management processes must be developed for many shrimp diseases (Cock et al. 2009).

Selected stocks have been demonstrated to perform significantly better in production than unselected populations for growth and disease resistance (e.g. Argue et al. 2002; Preston et al. 2004; Srisuvan et al. 2006). In general, genetic gains achieved through selected stocks appear to average about 5% per generation (Benzie 2009). The performance of given stocks in different environments appears to be similar where specific tests have been carried out, but these have usually involved environments that show few differences (different shapes of tanks or ponds). However, there is evidence for weak but significant genotype by environment (GxE) effects for *P. japonicus* in commercial ponds (Jerry et al. 2006b) and in tanks (Coman et al. 2004) and evidence of GxE interactions in *P. vannamei* at different stocking densities (Ibarra and Famula 2008). Strains improved in one program have performed well over wide geographical regions, but solid data on genotype by environment interactions in shrimp are still needed to determine whether regional strains will need to be developed.

These results have shown the power of genetic improvement in shrimp and provided information to determine the most efficient approach to future gains. Given the geographical and commercial scale of shrimp farming, and the fact that genetic work has been undertaken now for more than two decades in some of these species, there is still remarkably limited information published in this field.

Table 2. Heritabilities mean, (range) for selected characters in farmed penaeid shrimp. The data are means from the published literature. Sources additional to those listed in 1. Benzie (1998) are: 2. Kenway et al. (2006), 3. Jarayabhand et al. (1998), 4. Fjalestad et al. (1997), 5. Carr et al. (1997), 6. Pérez-Rostro and Ibarra (2003a), 7. Argue et al. (2002), 8. Gitterle et al. (2005a), 9. Gitterle et al. (2005b), 10. Gitterle et al. (2006a), 11. Gitterle et al. (2006b), 12. Hetzel et al. (2000), 13. Goyard et al. (2002), 14. Pérez-Rostro and Ibarra (2003b), 15. Ibarra et al. (2005), 16. Pérez-Rostro et al. (1999), 17. Castillo-Juarez et al. (2007), 18. Gitterle et al. (2006c), 19. Arcos et al. (2005), 20. Arcos et al. (2004), 21. Macbeth et al. (2007), 22. Ibarra et al. (2007a), 23. Huang et al. (2008), 24. Zhang et al. (2008), 25. Ibarra et al. (2009).

Character	Species				
	<i>chinensis</i>	<i>monodon</i>	<i>japonicus</i>	<i>stylirostris</i>	<i>vannamei</i>
<u>Growth</u>					
Weight measures	0.14	0.37 (0.05-0.56)	0.28	0.11	0.51 (0.00-1.42)
Length measures	(0.44-0.53)	0.29 (0.07-0.59)	-	1.03 (0.64-1.31)	0.71 (0.00-1.42)
Growth rate		0.51 (0.48-0.55)	-	-	0.69 (0.00-1.27)
<u>Survival</u>					
		0.53 (0.36-0.72)	-	-	0.05 (0.00-0.21)
<u>Diseases</u>					
TSV tolerance	-	-	-	-	0.31 (0.14-0.62)
WSSV tolerance	-	-	-	-	0.03 (0.00-0.21)
<u>Reproduction</u>					
Days to spawn	-	0.47	-	-	0.48 (0.41-0.54)
Egg number	-	0.41	-	-	0.13 (0.09-0.17)
Nauplii number	-	0.27	-	-	-
% Hatch	-	0.18	-	-	-
Number of spawns	-	-	-	-	0.20 (0.06-0.43)
Vitellogenin	-	-	-	-	0.29
hemolymph (adult)					
Egg diameter 1 st spawn	-	-	-	-	0.04 (0.00-0.07)
Egg vitellin 1 st spawn	-	-	-	-	0.38 (0.28-0.47)
Egg protein 1 st spawn	-	-	-	-	0.16 (0.13-0.18)
Egg acylglycerides 1 st spawn	-	-	-	-	0.18 (0.20-0.35)
Oocyte diameter (juvenile)	-	-	-	-	0.57
Oocyte diameter (adult)	-	-	-	-	0.23
Number of oocytes (juvenile)	-	-	-	-	0.00
Number of oocytes (adult)	-	-	-	-	0.11
Ovary maturity (juvenile)	-	-	-	-	0.71
<u>Stress</u>					
Resistance to hypoxia	-	-	-	-	1.44 (1.08-1.73)
Sources	23, 24.	1, 2, 3, 21.	12.	1, 13.	1, 4-11, 14-20, 22, 25.

Molecular and Genomic Resources for Shrimp

Molecular markers

Molecular variants have been used to assess the diversity and structure of wild stocks and domesticated populations for some time, and more recently to determine parentage in domesticated stocks and to develop genetic maps. Molecular genetics work on shrimp first focused on using molecular markers to assess levels of diversity in wild and cultured stocks and to assess population structure of wild stocks (Benzie 2000). The emphasis on wild stock structure reflected the strong fisheries influence on shrimp research at that time and the concern over levels of genetic variety in cultured stocks following production failures in early domesticated populations (Sbordoni et al. 1987; Sunden and Davis, 1991). The early studies used allozyme electrophoresis to assess variation at the protein level, but subsequent studies assessed variation in mitochondrial DNA and nuclear DNA using the latest tools as they became available, from random amplified polymorphic DNA (RAPDs), restriction fragment length polymorphisms (RFLPs) and microsatellites, to full sequence of DNA fragments and single nucleotide polymorphisms (SNPs). Today, a suite of nuclear or mitochondrial sequences from particular genes, intergenic sequences such as mtDNA control region are available for analysis depending on the specific question being addressed by the study.

The later DNA tools have generally proved more sensitive than allozymes and have shown evidence of a variety of genetic structures in wild shrimp populations. Some species show large geographical regions with high levels of gene flow, separated by sharp genetic disjunctions from other such areas. Other species show marked shifts in gene frequency over smaller geographical scales. The genetic disjunctions can either be related to large-scale biogeographical boundaries of considerable age, or to reflect present-day barriers to gene flow related to circulation patterns or differences in the temperature or salinity of water masses. More details on the structure of wild populations can be found in the reviews by Benzie (2000, 2009). However, key points with respect to discussion of domesticated shrimp are that molecular markers have demonstrated 1) the existence of species not distinguishable (or not easily distinguishable) using morphological characters (cryptic species) in *P. japonicus*, the earliest species farmed in the centre of its north-west Pacific range (Tsoi et al. 2005; 2007) and in *P. merguensis* in the centre of its range (Hualkasin et al. 2003); 2) major genetic differentiation between *P. monodon* populations from the south-west Indian and Pacific Oceans (Benzie et al. 2002; You et al. 2008); and 3) more subtle but significant genetic differences between *P. vannamei* populations from different parts of its natural range in the east Pacific (Valles-Jimenez et al. 2005, 2006). These results suggest the possibility of local adaptation in shrimp populations, the possibility of genotype by

environment interactions, the need for care in choosing the source(s) for establishing domesticated populations and possible issues for interactions between domesticated and local stocks.

An immediate application for molecular markers was their use to assess levels of genetic diversity in domesticated stocks. This showed that marked declines in the reproductive performance of *P. japonicus* were associated with reductions of molecular genetic diversity indicative of considerable inbreeding (Sbordoni et al. 1986, 1987). Since then, a number of studies have shown marked reductions in genetic diversity in cultured populations (reviewed in Benzie 2000 and Benzie 2009). However, no decline in genetic diversity was reported for stocks of *P. chinensis* (Zhang et al. 2004) and *P. vannamei* (Cruz et al. 2004; Soto-Hernandez and Grijalva-Chon 2004; Luvesto et al. 2007; Lima et al. 2008; Perez-Enriquez et al. 2009). Therefore, not all reductions in levels of molecular variation are associated with declines in production performance, and there is only one report of a positive association of DNA heterozygosity with individual performance in inbred *P. stylirostris* populations (Bierne et al. 2000). The genetic diversity in domesticated stocks obviously depends on the original variation in the founder stocks and the nature of the regime used to manage the population. It is also clear that the overall level of molecular diversity, measured using a relatively small number of loci (as is usual) provides only a general guide as to the diversity in the cultured population and is not necessarily directly linked to variation at the often polygenic morphological characters of importance in genetic improvement.

Parent Assignment

The avoidance of the deleterious consequences of unintended inbreeding demanded the ability to avoid inbreeding of close relatives. This ability is not provided by population level assessments of molecular variation, but by managing mating between parents. It was then a short step to using molecular markers to assist parent assignment in cultured populations, particularly once highly variable markers such as microsatellites, became available. Panels of microsatellite markers have been developed for *P. chinensis* (Dong et al. 2006), *P. japonicus* (Sugaya et al. 2002; Jerry et al. 2004, 2006b), *P. monodon* (Jerry et al. 2006a; Li et al. 2007) and *P. vannamei* (Alcivar-Warren et al. 2003). In general, these have shown that sufficient variation is provided by approximately 10 loci to allow 99% accuracy of assigning parentage to shrimp sampled from a mixed population. The levels of relatedness of the sampled shrimp can also be assessed and allow breeders to avoid mating individuals that are too closely related. The markers also allow for traceability of stocks through production chains, thereby increasing confidence in food security (Maldini et al. 2006). These methods have been used as the primary assessment of consanguinity in populations of *P.*

japonicus and *P. monodon* in Australia subject to mass selection in order to avoid inbreeding (Preston et al. 2009). More commonly, these methods appear to be used as backups where physical tagging is used preferentially to track pedigrees.

Functional genomics and candidate genes

Relatively few shrimp genes had been characterized more than ten years ago and DNA sequences were limited (Benzie 1998), but increasing numbers of sequences and gene characterizations have been published in the last decade (Benzie 2009). The hundreds to thousands of sequences available at the end of the 20th century can be compared with the hundreds of thousands available today. There is also an increasing number of genomic tools including BAC libraries for *P. vannamei* (Zhang et al. 2010), *P. monodon* (Wuthisuthimethavee et al. 2009) and *P. japonicus* (Koyama et al. 2010), and FOSMID libraries for *P. monodon* and *P. vannamei* (Saski et al. 2009). Development of markers a few years ago was characterized by processes resulting in less than ten to hundreds of markers (e.g. microsatellites: Vonau et al. 1999; Moore et al. 1999; Cruz et al. 2002; Meehan et al. 2003; Glenn et al. 2005) but now, use of advanced techniques of DNA marker development and the plethora of sequence data coming from next-generation DNA sequencing platforms (i.e. Roche 454 FLX, Illumina GIIx) can result in hundreds to thousands of potential DNA markers being rapidly isolated (e.g. O'Leary et al. 2006; Gorbach et al. 2008; Ciobanu et al. 2009).

A detailed review of the genes being characterized for shrimp is beyond the scope of this paper, but studies have tended to focus on genes that might be related to response to bacterial (e.g. Destoumieux et al. 2000; De Lorgeril et al. 2005; Amparyup et al. 2010) and viral (e.g. He et al. 2005; Pan et al. 2005; Wang et al. 2006, 2008; Prapavorarat et al. 2010) diseases or both (Gross et al. 2001; Robalino et al. 2007; Wang et al. 2008; Ma et al. 2008), reproduction (see reviews by Lo et al. 2007; Ibarra et al. 2007b), growth (e.g. Lyons et al. 2007) and a number of other genes that have attracted the interest of particular researchers (e.g. Zhang et al. 2007b). Around the year 2000, conference presentations were focused on providing a list of possible gene identities from searches of DNA data bases using shrimp sequences (e.g. BLAST searches) (Benzie 2005). Since then, more than a hundred papers have been published where genes have been sequenced, their amino-acid sequence inferred and corroborated and their expression in one or more tissues and/or in different environments assayed. In the case of antimicrobial activity, a substantial database has been set up (Gueguen et al. 2006). However, there are no publications yet, where any of these possible candidate genes are finely mapped or where they are used in selection programs. Targeted approaches to understanding biochemical or metabolic pathways have not yet emerged, although work in disease response and reproduction is moving in that direction.

Functional genomics is being undertaken using species of shrimp that are commonly farmed, but results are still fragmented.

Genetic Maps

First order genetic maps exist for only four of the main domesticated species: *P. chinensis*, *P. japonicus*, *P. monodon* and *P. vannamei* (Table 3). These maps are largely based on AFLP markers and the spacing of markers simply reflects the number of markers mapped, with more recent studies generally mapping more markers than earlier studies for a given species.

The number of linkage groups observed is now approaching that expected from the chromosome counts (Chow et al. 1990) for most species. The advantage of AFLP markers is that they can provide a large number of markers quickly and cheaply, but the markers vary from species to species, even family to family and cannot be used for comparative genomics. More recently, maps using microsatellites (You et al. 2010) or SNPs (Du et al. 2009) as the main markers have been published for *P. monodon* and *P. vannamei* respectively. These provide a crude basis for comparative mapping but to date few markers have been used in common. Marker density is still sparse in even the densest of shrimp maps (around 500 markers relative to thousands or millions in vertebrate maps). This lack of depth means there is no knowledge currently on genome structure such as rates of recombination across the genome, haplotype blocks and so on, that are needed for the current maps to be practically useful in breeding programs. There is a need to increase the density and coverage of the shrimp genome using type I genetic markers (microsats, SNP) to allow detailed comparative mapping and provide the capability to undertake effective genome-wide selection.

The maps developed to date have largely mapped molecular markers alone and few have included phenotypic characters, although sex has been scored in three species with associations to single linkage groups in each case: *P. vannamei*, (Zhang et al. 2007a; Alcivar-Warren et al. 2007; Du et al. 2009), *P. japonicus* (Li et al. 2006a) and *P. monodon* (Staelens et al. 2008). Putative QTLs have been identified for growth in *P. chinensis*, *P. japonicus*, *P. monodon* and *P. vannamei*, but the lack of marker density means these are not well defined. The level of linkage indicates the distance between the marker(s) and the locus of interest spans several million base pairs and that much more work needs to be done to identify the candidate genes. A quantitative trait locus associated with growth has been reported for *P. japonicus* and a possible candidate gene with a role in fatty acid metabolism, *ELOVL-MJ* identified (Lyons et al. 2007). However, much more work needs to be done to establish whether *ELOVL-MJ* is responsible for the observed QTL effects. Only one effective marker for a phenotypic

trait has been developed for any shrimp species as yet and that is for sex in *P. monodon* (Staelens et al. 2008). This marker was used by You et al. (2010) to identify their linkage group 26 as the W chromosome in their *P. monodon* study, but the site was not variable and could not be used to distinguish sex in a *P. vannamei* mapping study (Du et al. 2009).

Table 3. Summary data on the genetic maps for penaeid shrimp. Number of maps refers to the total number of combined sex (c), female (f) or male (m) maps reported. More detailed information is given for the most detailed map available for the species with the data given for combined sex, female and male maps in that order. The source for the more detailed information is given in italics in the last column of the Table, with other sources given in regular font. The markers used for the map for which details are listed are given in italics in column three. Where there is more than one map that has advanced features not shared by the others, separate data is given in a row below. Sources are 1. Li et al. (2006b), 2. Moore et al. (1999), 3. Li et al. (2003), 4. Wilson et al. (2002), 5. Maneeruttanarunroj et al. (2006), 6. Staelens et al. (2008), 7. Pérez et al. (2004), 8. Li et al. (2006a), 9. Wuthisuthimethavee et al. (2005), 10. You et al. (2010), 11. Yue et al. (2004), 12. Sun et al. (2008), 13. Tian et al. (2008), 14. Zhang et al. (2007a), 15. Alcivar-Warren et al. (2007), 16. Du et al. (2009).

Species (Haploid chromosome number)	No of maps	Type of markers (and total number mapped by all studies)	No. of markers mapped c, f, m	Average space between markers (cM) c, f, m	% of genome mapped c, f, m	No of linkage groups c, f, m	Sources (time span of publication)
<i>P. chinensis</i> (44)	11 c,f,m	<u>AFLPs</u> (638+) Msats (36) RAPDs (237)	-, 197, 194	-, 13.5, 11.0	-, 74, 73	-, 35, 36	1, 11, 12, 13 (2006-2009)
<i>P. japonicus</i> (43)	5 c,f,m	<u>AFLPs</u> (865)	-, 139, 245	-, 7.8, 8.3	-, 44, 88	-, 33, 43	2, 3, 8 (1999-2006)
<i>P. monodon</i> (44)	17 c,f,m	<u>AFLPs</u> (1817) Msats (12) EST-SSRs (36) EPICs (1) SSCPs (6) SNPs (1) SCAR (1)	-, 405, 547	-, 4.6, 4.1	-, 92, 113	-, 45, 42	4, 5, 6, 9, 10 (2002-2010)
		<u>AFLP</u> (85) <u>Msat</u> (347)	-/, 36/171, 49/176	-, 13.8, 11.2	-, 63, 68	-, 36, 37	10
<i>P. vannamei</i> (43)	6 c,f,m	<u>AFLPs</u> (<u>Msats</u> (43) EST-SSRs (5) SNPs (418)	-/, 319/18, 252/14	-, 15.1, 14.5	-, 88, 90	-, 45, 45	7, 14, 15, 16 (2004-2009)
		<u>SNPs</u> (418)	418, -, -	5.4, -, -	43, -, -	45, -, -	16

The principal finding of this review of recent progress is that an increasing amount of molecular genetics work is being undertaken in shrimp, including marker development, gene characterization and functional genomics. These tools are being used to increase understanding of physiology, growth and response to disease in shrimp. However, as far as utilizing marker assisted selection, whole genome selection or identification and use of candidate genes for marker assisted selection to meet the potential of molecular tools to provide more efficient means of selecting improved strains, work has just begun.

Discussion

This paper has provided brief reviews of key components of genetic improvement in shrimp aquaculture as they relate to assessing the extent to which quantitative and molecular genetics are integrated in shrimp aquaculture production. The results have shown that some quantitative genetic information is available for several shrimp species, but that these data are still limited in extent and sophistication. Despite the examples of the value and practice of genetic improvement programs available in agriculture, shrimp production has been driven by short-term economic factors and domesticated stocks have been developed from necessity, rather than through strategic planning. The application of quantitative genetic methods has been primarily through public sector research in the first instance, although work was conducted often with individual industry members. These have led to more structured approaches to genetic improvement through the use of pedigreed populations and the use of quantitative genetic methods by the major genetic improvement companies now operating in the shrimp industry.

Similarly, the use of molecular tools has been pioneered by the research community and public sector; first, to assess the genetic structure of wild stocks and second, to assess the genetic diversity in cultured stocks. Investigations of wild stocks demonstrated considerable structure including the existence of cryptic species. They have not resulted in any particular change in approach by the commercial industry, although domesticated stocks that have been established more recently have deliberately accessed more diverse wild stocks. However, this decision was more likely to have been affected by the finding of reduced molecular variation in stocks established from small founding populations, and the concerns for deleterious levels of inbreeding. Although molecular markers have proved to be effective in tracking parentage in cultured populations, there are still questions concerning their cost and their application in the industry is limited to programs supplying less than 1% of improved stock worldwide. Pedigreed populations are usually tracked by rearing families in separate containers and by tagging them at later stages of development.

The isolation and characterisation of an increasing number of individual shrimp genes is being reported, but more concentrated research on biochemical or physiological pathways, despite the consolidation of work in reproduction and disease response has not yet been achieved. Work to better understand the molecular basis for shrimp cellular and physiological function is in progress but there are still few candidate genes identified. Anonymous molecular markers have played a great role in developing genetic maps for shrimp, but almost all of the characters mapped are molecular markers. Only sex determining regions have been mapped in a couple of shrimp species and a few regions indicating relatively large chromosomal regions as ill-defined QTLs for growth identified. In that sense, integration between quantitative and molecular genetics is being undertaken, but is still limited. The majority of this work is also undertaken largely by public sector research, although private sector involvement is growing. The best shrimp maps developed have relatively few molecular markers mapped (<500) when compared with terrestrial livestock and plants (with several thousand to millions of markers mapped), and few associations with morphological, physiological or metabolic traits have been established.

The relatively short time over which industrial shrimp aquaculture has developed means that other information which is available for agricultural organisms is lacking for shrimp. First, the extent and depth of information on the biology, physiology, metabolism and biochemistry of shrimp is far less than that for cattle, pigs, chickens or finfish, for example. Second, the amount of information from pedigreed data sets is far less in spatial and temporal extent and, because of the lack of primary biological information, covers a relatively limited number of traits. However, domestication programs have existed for some 20 years and the relative paucity of quantitative and molecular data also reflect a lack of strategic approach by the industry and the limited investment in genetics in the sector.

The advantages of integrating molecular and quantitative genetics are clear, and the directions of the research community reflect these. The ability to translate these technological achievements into competitive commercial outcomes is harder, made more so by the limited investment and limited information available in the sector. Moves to integrate quantitative and molecular genetics in shrimp genetic improvement are being undertaken, but the limited scale of activity slows this process and their integration into industrial activity.

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