

Aquaculture in the Asia-Pacific Region: Applications of Molecular Population Genetics

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ABSTRACT

The utility of applied molecular population genetics in aquaculture has been questioned by some quantitative geneticists. They argue that there have been few examples where research in this area has directly resulted in development of improved strains used in culture. Here I suggest that this could be a narrow view of the diversity of issues that need to be addressed when new stock improvement programs for aquatic species are initiated. My belief is that this view has arisen due to fundamental differences in the relative starting points faced when new productive strains of terrestrial vs aquatic farmed species are developed. Aquatic species in general, possess very high fecundities and as a consequence of husbandry practices are therefore prone to lose genetic diversity much more rapidly in culture than comparable terrestrial farmed species. Additionally, natural populations of most aquatic farmed species are still common in the wild, unlike their terrestrial counterparts, and so can provide important genetic resources that can be exploited in sustainable ways to improve culture lines. Taken together, this implies that genetic diversity is a much more significant issue broadly speaking, for farmed aquatic species compared with terrestrial farmed species. Thus farming of aquatic species can benefit from application of well-conducted molecular population genetic research. Thus, the narrow view that some quantitative geneticists have taken on the utility of molecular population genetics in aquaculture, in my opinion may miss the broader applications of the technologies that can assist a move to development and farming of improved breeds of aquatic species while conserving natural gene pools in the Asia-Pacific region.

Keywords: Aquaculture, Asia-Pacific Region, aquatic species, genetic diversity

INTRODUCTION

Aquaculture is one of the fastest expanding industries worldwide and nowhere in the world is this more apparent than in Asia, a region that currently accounts for around 80% of total world production. The industry will continue to expand and aquaculture will be of growing importance for regional food security and national development in the Asia-Pacific region. While most farmed aquatic species in the region have yet to be improved, the trend in America and Europe is to move from farming essentially wild animals to production of genetically-improved, high performing aquatic breeds. In many parts of the world, the industry now focuses more and more on quantitative genetic approaches to improve the productivity of cultured species.

This development mirrors advances that have occurred in terrestrial farmed species over the last 60 years where significant advances have been made to the relative productivity of the major terrestrial species we produce in agriculture. For example, genetic improvement programs have; improved meat production in chickens by up to 200%, increased milk production in dairy cows by over 150% and meat production in pigs by around 100%, since 1940 (FAO, 2006). This rapid development follows approximately 10,000 years of domestication and genetic improvement of these species by humans, to a point where modern farmed terrestrial animals are physically unlike their now extinct wild relatives. In contrast, with a few notable exceptions, most farmed aquatic

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species are essentially wild organisms brought into husbandry. The future however, will be a move by industry to farming improved lines with traits often very different to phenotypes that are favoured in the wild.

When we examine the genetic characteristics of most farmed aquatic species, it becomes obvious very quickly, that most stocks have been developed with little regard for genetic attributes that would increase their value as farmed stocks. On closer inspection, levels of inbreeding are often high and as a consequence, genetic variation is generally low. These factors are often compromised by the husbandry practices we employ in culture that further erode the remaining genetic variation across generations in culture. As a consequence, many culture lines may have limited ability to respond to breeding programs when the industry moves to stock improvement (Frankham *et al.*, 2002 ; Allendorf and Luikart, 2007). Unintentional selection by animal breeders and farmers that results from husbandry practices can also compound the problem leading to fixation of unfavourable phenotypes. A widely acknowledged example is rapid declines in size at sexual maturation in female giant freshwater prawn (*Macrobrachium rosenbergii* - GFP). A characteristic of most aquatic farmed species, that has not been well understood, is the fact that many are very prone to rapid loss of genetic diversity, much more so, than are equivalent farmed terrestrial animal species.

Most aquatic farmed species are highly fecund with females capable of producing thousands if not hundreds of thousands of offspring from a single mating. This is not generally a problem in the wild where larval and juvenile survival is generally very low and populations and breeding numbers are generally high. In culture however, relative survival of offspring per mating can be very high and number of broodstock used to produce larvae and fry are often very limited. So the genetic relationship between surviving offspring can be very high, and over generations in the hatchery, is likely to increase significantly. This issue is often compounded by the fact that most broodstock used to produce the offspring for growout are unlikely to have been collected in a systematic way and the cost of collecting new broodstock and their maintenance over time, can result in their reuse and cross generational mating,

leading to compounding levels of inbreeding across generations. This problem is often not considered, or is not obvious to hatchery managers, because breeding registers are seldom employed. Across generations inbreeding levels are likely to increase and genetic diversity will decline potentially compromising future response to stock improvement programs.

High levels of inbreeding and low genetic diversity in many aquatic cultured stocks in comparison with their wild relatives does not necessarily mean that artificial selection programs are likely to fail. In fact, where aquatic species have been subjected to stock improvement programs, response to selection can be quite dramatic and it is not unusual to see very rapid genetic gains in excess of 10 or 20% per generation achieved. This is much faster than can be achieved for most farmed terrestrial species (TFAS) where genetic gains of 3 to 5% per generation are considered very good. This is because even given low genetic diversity and high levels of inbreeding relative to their wild cousins, genetic diversity levels in most cultured aquatic stocks are still orders of magnitude higher than equivalent TFASs, simply because they have spent many fewer generations in culture. The problem remains however, as most AFASs have greater potential to lose any remaining genetic diversity very rapidly, much faster than TFASs, if it is not managed carefully.

As interest has grown in many parts of the world to advance aquaculture from essentially farming wild animals to farming improved, domesticated breeds, both quantitative geneticists/animal breeders and population geneticists have become involved in this development. In particular, research by population geneticists has focussed on developing and applying molecular genetic marker analyses to assay genetic diversity in cultured aquatic species for a variety of applications. In recent times however, this type of study has been questioned by some quantitative geneticists working in developing countries and who gained their experience in stock improvement programs on terrestrial animal species, as being wasteful of limited resources for research. They argue that the majority of productivity gains in agriculture (and now, aquaculture) have come directly from animal breeding programs not molecular diversity studies. Thus, they have questioned the

relevance and value of molecular diversity research in aquaculture. While it is true that, in some instances, significant resources have been largely wasted in poorly-designed and conducted molecular diversity projects, particularly in the developing world in recent years, it is my view, that criticisms of the approach ignore some fundamental differences between the relative starting points for genetic improvement programs in agriculture and aquaculture. The focus of the current paper is to highlight these issues and to identify a more productive approach to future programs.

When we compare the general situation that faces aquaculture with that in terrestrial agriculture as they relate to issues relevant to a move to farming improved breeds, immediately it is obvious there are some stark contrasts. While humans have been changing the attributes of farmed terrestrial animal species for thousands of years, the number of farmed terrestrial animal species is quite small (~6 to 10 species worldwide) compared with more than 100 AFASs that are either farmed or are being trialed in aquaculture today. Secondly, the wild relatives of most TFASs have been extinct for 1000s of years while healthy populations of most AFASs still exist in the wild. Thus, available wild genetic resources for most AFASs are extensive and largely unexploited, but ongoing genetic improvement of TFASs must depend on the limited genetic resources that remain in cultured stocks after many generations of inbreeding. While both TFASs and AFASs could benefit from transgenic options in the future, consumer acceptance issues are likely to remain a problem with this technology. Apart from the issues identified above, a major contrast will always be the fact that major life history traits in AFASs are usually quite different to those present in TFASs. Of particular relevance here, are differences in relative individual fecundity, a factor that seems to have been largely ignored by quantitative geneticists except when they benefit from the large number of offspring that can be produced from a very small number of breeders in stock improvement programs. For TFASs this has never been a problem, but for aquatic species it can be a genetic 'time-bomb', that can rapidly erode away any remaining genetic diversity including the important exploitable component. This is where

I believe, applied molecular population genetics, when used appropriately, has a major role to play in the development of modern aquaculture. Of specific relevance here, the science of molecular population genetics can provide important data that will assist with (1) better broodstock choice, (2) monitoring genetic diversity across generations to reduce inbreeding levels, (3) reducing effects of unintentional selection and help to maintain the selection response in a breeding program. In addition, molecular diversity studies can monitor and help to maintain healthy effective population size in the hatchery and assist biologists to better understand the reproductive biology and social traits of target species. Finally, modern genomic approaches can identify critical genes and even individual mutations that influence important quantitative traits and hence focus the attention of animal breeders on critical areas of the genome. Below I provide some examples where I believe molecular population genetic research has contributed to the productivity of cultured aquatic species.

Applications of Molecular Population Genetics in Broodstock Selection

Wild genetic resources of most cultured aquatic species are still extensive but are often poorly known. Most farmers source their broodstock from other breeders potentially compounding genetic diversity problems at the start of any stock improvement program. Knowing the genetic resources that are available in the wild and their relative levels of genetic diversity can allow breeders to make informed choices to optimise the diversity available to future breeding programs. These data can also assist in assessing the potential for obtaining hybrid vigour vs outbreeding depression outcomes in crosses made between genetically discrete stocks and for developing 'synthetic culture lines' that are genetically compatible but which possess high genetic diversity due to their divergent origins (an example is the 'GIFT' strain of Tilapia). Equally, genetic diversity studies of wild and cultured lines provide a reference point for assessing impacts that husbandry practices may have had on genetic diversity levels in cultured stocks, before inbreeding becomes a significant issue for farmers.

Case Study: Giant Freshwater Prawn (M. rosenbergii) Culture in Asia

GFP culture is the 6th largest culture industry in Asia and is worth close to USD 1 billion currently per year to the region. While most farmers in Asia have sourced their broodstock from local wild stocks in the past, the industry was initiated originally from 12 individuals from Malaysia in the 1970's and taken to Hawaii where the life cycle was closed and hatchery technologies developed. This stock was later translocated widely outside Asia to develop new culture industries in regions where the species is exotic. GFP are now cultured in over 43 countries worldwide on five continents. Genetic diversity levels in all stocks outside Asia are likely to be low however, due to the extreme bottleneck that the original introduced population was exposed to when the original Hawaiian culture line was developed. Impact of repeated translocations of this stock to new culture locations has most probably compounded this problem. In contrast in Asia, while local wild stocks were sourced for broodstock during development of local culture industries, this was done basically without knowledge of genetic diversity levels or a clear understanding of why or how to maximise diversity in culture. More recently, most new farms source their culture stock from existing farms and it is also common for only small numbers of breeders to be used in the hatchery. Thus genetic diversity levels in the Asian culture industry are also likely to have been impacted by the process of culture industry development and inbreeding levels may be high. This could explain the observation reported in many locations, that female size at sexual maturation has declined over years in culture, a sure sign that unintentional selection and inbreeding have resulted in early maturing, small-size females being sourced repeatedly as broodstock. Much of this has remained unstudied with the exception of a very recent paper by Chareontawee *et al.* (2007) on Thai GFP stocks.

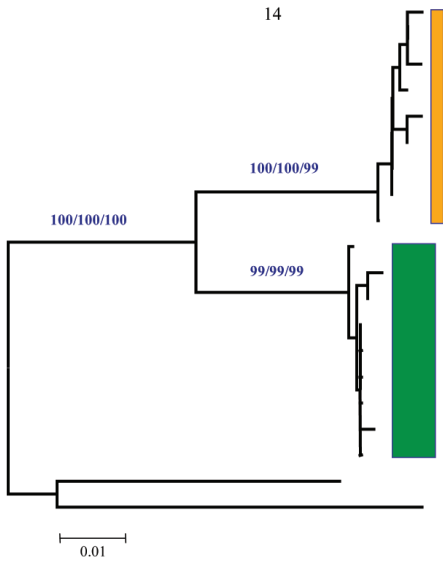
Currently, there is growing interest in many countries for stock improvement programs for GFP, but if inbreeding levels are high, any response to selection could be compromised by low exploitable levels of genetic variation. Thus, developing a better understanding of genetic diversity in culture lines and their wild antecedents, will allow informed choices to be made about initiating new culture lines, high in

genetic diversity, prior to stock improvement programs being initiated that may be compromised by low exploitable levels of diversity.

From a theoretical population genetic perspective, when life history characteristics of GFP (primarily freshwater with a short estuarine phase, life cycle confined to stream systems) are considered in the light of the extensive natural distribution of the species, levels of genetic diversity in wild stocks are likely to be high and may be structured, geographically. The natural distribution of GFP includes from Pakistan in the west across southern and SE Asia to central Vietnam, includes some Philippine islands and northern Australia, PNG and extends to some larger Pacific islands. Until recently nothing was known however, about genetic diversity in either wild or cultured populations of GFP. Recent studies by de Bruyn *et al.* (2004a ; 2004b ; 2005), resolved the patterns of genetic diversity in wild stocks of the species and this baseline data form a foundation for future comparative analyses of culture lines.

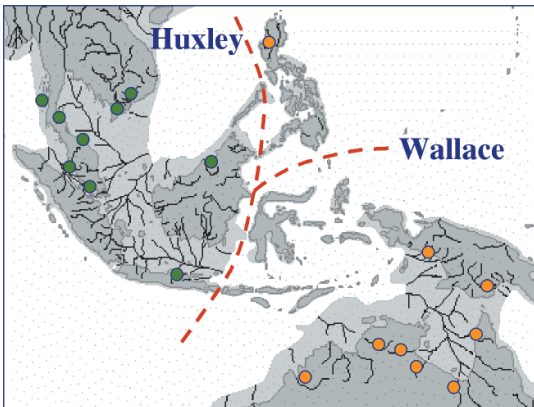
Taxonomists currently consider wild GFP populations to be monophyletic across the species' extensive natural range. Molecular analysis of broad-scale variation in wild stocks using the highly conserved 16S RNA mtDNA gene however, identified two major divergent lineages (*Fig. 1a*) referred to as 'eastern' and 'western' forms with distributions divided by Wallace's Line (extends to Huxley's line to the north – *Fig.1b*) (de Bruyn *et al.*, 2004a). A calibration of the theoretical time of separation (MRCA) of the ancestors of the two forms was 5 to 12 million years bp, indicating that the two lineages probably diverged in the Miocene, yet modern populations of the two different forms cannot be, or are difficult to, distinguish morphologically.

Analysis of diversity within the two major wild GFP lineages using a more rapidly evolving mtDNA gene (CO-I) identified additional, fine-scale geographic population structure. Within the 'western' or Asian lineage, a major genetic break was evident between wild populations north and south of a biogeographical zone, referred to as the 'Isthmus of Kra' in southern Thailand, an area of low topography that was inundated a number of times during sea level changes (eustasy) over the last 100,000 years (*Fig. 2* - de Bruyn *et al.*, 2005). Fine-scale population



(a)

Fig. 1a, b: Neighbour-joining tree of molecular diversity in the mtDNA 16SRNA gene in wild stocks of *M. rosenbergii* showing deep divergence between 'eastern' and 'western' populations



(b)

Fig. 1b: The geographic pattern of 16SRNA gene diversity in wild stocks of *M. rosenbergii* from across the species natural range related to two major biogeographic regions

structure within the 'eastern' form was greater with five divergent lineages identified, four of which occur in northern Australia and that have distributions correlated with known biogeographical regions (Figs. 3a and 3b - de Bruyn *et al.*, 2004b). When the analysis was extended to include nuclear markers

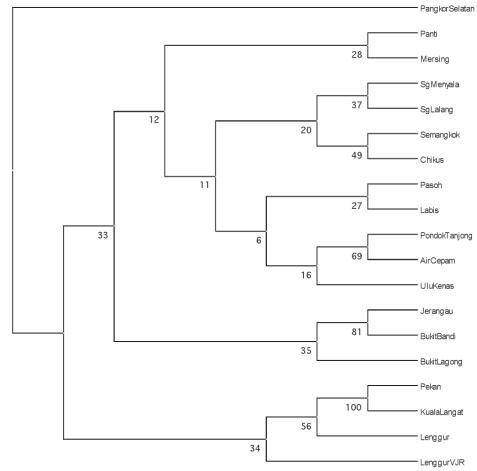
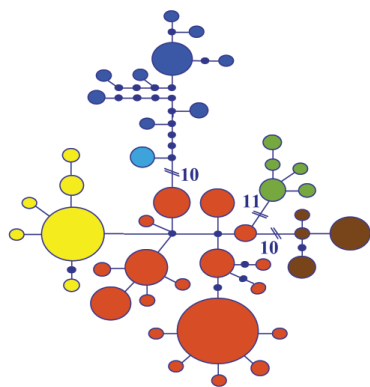


Fig. 2: Patterns of diversity in the 'western' form of *M. rosenbergii* at the mtDNA CO-1 gene showing a break concordant with the Isthmus of Kra' region in southern Thailand

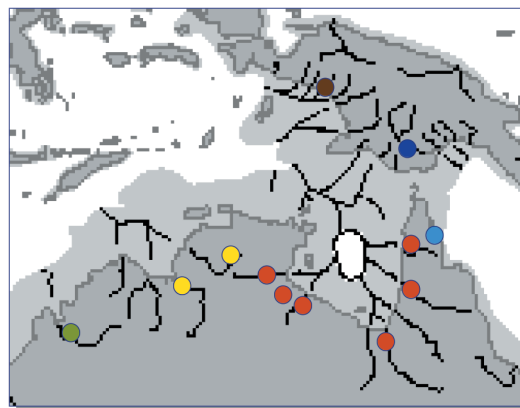
(microsatellites) for the 'eastern' form, populations were structured at a similar regional scale to that identified with the fast-evolving mtDNA gene marker indicating that sets of specific nuclear alleles were restricted to the different geographical clades Figs. 4a and 4b). If this is true for neutral, non-coding alleles at microsatellite loci, there is a strong chance that local selection and genetic drift will also have resulted in unique alleles at coding loci, perhaps even loci that may influence quantitative traits affecting productivity.

Data on patterns of wild stock diversity have relevance for the GFP culture industry because the patterns imply that to date, very little of the natural genetic diversity present in GFP wild stocks has been captured in culture (de Bruyn *et al.*, 2004a; 2004b; 2005). Of the diversity that has been captured, it is likely that a significant proportion may have been lost or eroded by high levels of inbreeding due to culture management practices. Given the extent of diversity revealed, potential for all possibilities from heterosis to outbreeding depression may be possible in crosses between discrete wild gene pools. Outbreeding depression can result from the mixing of incompatible genomes and can lead to poor outcomes in the progeny. For example, if outbreeding produces genetic incompatibilities, offspring may not survive, may be infertile or have low fertility relative to inbred progeny or may have adaptive incompatibilities



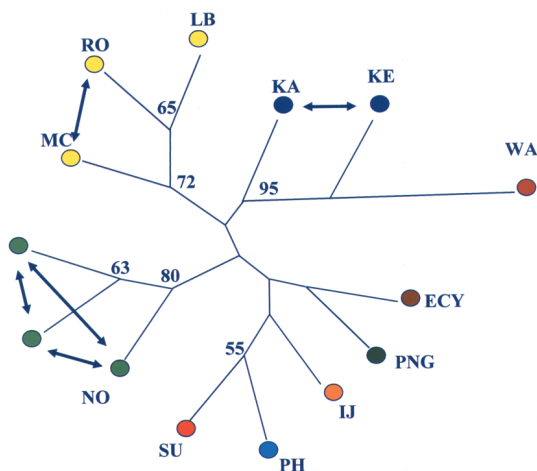
(a)

Fig. 3a: Minimum spanning network of CO-I haplotypic diversity in the 'eastern' form of *M. rosenbergii* indicating the distributions of four divergent clades across northern Australia



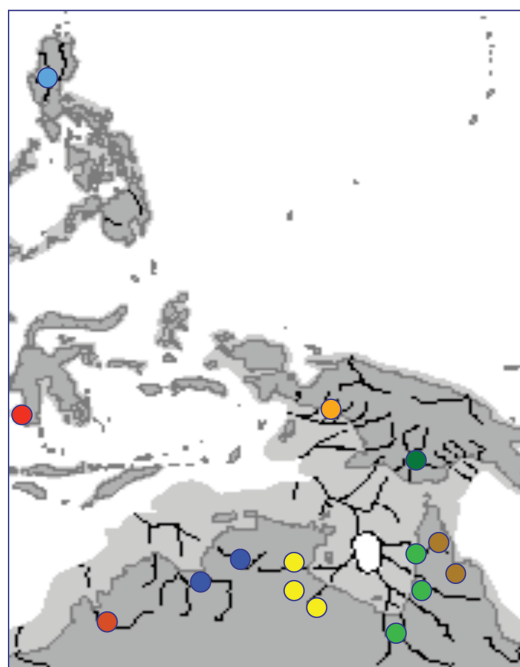
(b)

Fig. 3b: Patterns of diversity in the 'eastern' form of *M. rosenbergii* across northern Australia and PNG at the mtDNA CO-I gene



(a)

Fig. 4a: Neighbour joining tree of microsatellite variation in the eastern form of *M. rosenbergii*



(b)

Fig. 4b: The pattern of geographic variation in microsatellite diversity in 'eastern' populations of *M. rosenbergii* are concordant with mtDNA clades

leading to low relative fitness. Unfortunately, potential for outbreeding depression is impossible to predict but in general terms, the more genetically divergent parents are, the higher is the probability of producing poor outcomes in the progeny. Since GFP have been translocated widely for culture and molecular analysis has indicated that the 'eastern' and 'western' forms probably last shared a common ancestor greater than 5 million years b.p., care should be taken in mixing GFP populations either for culture or when culture stocks escape to the wild. Even within the two major GFP

lineages, a conservative view should be adopted when crosses are considered for culture or stocks are translocated to new locations where wild stocks are still healthy.

Recent molecular diversity studies of GFP wild stocks provide data that allow preliminary predictions about potential for genetic compatibility between different wild GFP stocks and hence provide a geographical scale at which 'synthetic' culture lines might be developed. These data could form the basis for a new systematic approach for broodstock collection and a breeding program, particularly for the Asian region designed to produce a highly genetically diverse base population for a future culture stock improvement. Further work will be needed at fine spatial scales however, to better understand the significance of population divergence and to assess whether some local variation may be clinal or reflect isolation and independent evolution in the recent past.

A number of studies have been initiated recently with collaborators in the region to explore some of the practical applications of the GFP molecular diversity data. In Vietnam, we are trialing a diallelic cross among two genetically-discrete wild Vietnamese GFP strains and a third culture strain from Thailand, to relate genotype to strain performance and potential for crosses to show hybrid vigour. A new project will estimate relative levels of inbreeding in specific culture lines and the wild populations from which they were derived to quantify the impact that past management practices have had on genetic diversity in culture lines in the region. In late 2007 we plan to introduce new culture strains of GFP from Asia to the Pacific region under quarantine to assess their relative performance against the 'Hawaiian' strain that has been cultured there, and more widely around the world, since the 1980's, but that has showed some decline in productivity.

Applications of Population Genetics Studies on Genetic Diversity in the Hatchery

A number of recent molecular studies have reported significant declines in genetic diversity in hatcheries. The fact that this has been reported in diverse species from marine fish to mollusc species suggests that the phenomenon may not be uncommon. Factors identified as potentially contributing to this problem include; non-systematic choice of broodstock, use of small numbers of parents, unequal sex ratios in the parents, social factors in communal broodstock tanks (e.g. dominance hierarchies) leading to unequal contributions to fry and variation in

family survival. Any of these factors in isolation or in combination, can significantly erode genetic diversity in the fry and rapidly increase inbreeding rates. Understanding the causal factors will be very important for sustaining culture productivity and can help with the design of better breeding strategies. Molecular diversity studies of specific species can provide these data and have been used to identify the likely causal agents.

A study by Hara and Sekino (2003) on Japanese flounder (*Paralichthys olivaceus*) using parentage assignment based on microsatellite genetic markers demonstrated that only 57% of hatchery broodstock spawning in communal tanks contributed to offspring. This resulted in a 29% loss in allelic diversity in the offspring compared with their parents and average heterozygosity was also significantly reduced. They observed that this was happening every hatchery cycle and so, even if genetic diversity had been relatively high in the parents at the start, it can be eroded very rapidly across generations. Since loss of genetic diversity is essentially random, even quantitative alleles that confer high fitness can be lost by this process. Another recent study of Sea Bass (*Lates calcarifer*) by Frost *et al.* (2007), reported that 55% of progeny were sired by a single male when 7 males were used in a communal breeding tank and whole families did not survive to fingerling stage. Family loss was apparently random and unrelated to relative growout performance, implying that even high performing families could be lost by chance. In addition, the effective population size (EPS) of the offspring was only ~ 50% that of their parents, implying that only a limited number of potential parents contributed their genetic diversity to the fry. Size-grading of the fry prior to stocking, can apparently further erode genetic diversity levels. Thus, hatchery practices can have major impacts on genetic diversity in offspring and significantly increase inbreeding rates over very few generations. The same outcome is unlikely to occur in terrestrial animal species due to major differences in life history traits. This problem has been largely ignored in most fish, mollusc and crustacean hatcheries until very recently, yet farmers often comment on losses in productivity in their culture stocks over time.

Molecular population genetic studies can also have an important role to play in assessing the impacts that hatchery genes may have on

wild gene pools in aquatic species. This is not a significant issue for farmed terrestrial animal species because wild gene pools for most species are long extinct. It is obvious that for aquatic species produced in hatcheries however, that accidental escapes to the wild or deliberate releases for stock enhancement or ranching purposes have the potential to lead to contamination of wild gene pools with hatchery genes. Since most hatchery-produced stock are less genetically diverse than their wild counterparts, when interbreeding occurs, levels of inbreeding are likely to increase and genetic diversity to fall consequently, in the mixed population. Even traits that are favoured in the wild may be lost by chance, as has been reported recently in salmon. Vasemagi *et al.* (2005) used molecular markers to identify interference by hatchery-reared fish that had been stocked in wild rivers in Europe on natural spawning of wild Atlantic salmon (*Salmo salar*) strains. As we produce more aquatic species in culture and stock enhancement is practiced more widely to replenish declining wild populations, it will be important not to compromise the fitness of wild adapted populations. Molecular population genetic analyses provide a powerful set of techniques for monitoring stocking impacts and to assess the extent of introgression of hatchery genes into wild gene pools.

Thus integrating research on molecular population genetics, quantitative genetics, animal breeding and nutrition provide the best option for new stock improvement programs on aquatic species. While to date, an integrated approach has been employed on only a relatively few species, where it has been practiced, outcomes have been very encouraging (e.g. Tilapia, Atlantic salmon, Pacific salmon and Channel catfish). Nile tilapia (*Oreochromis niloticus*) is probably one of the few species cultured widely in Asia that has benefited from such an approach to stock improvement. While Nile tilapia has been cultured in Asia for decades, declines in stock productivity due to high inbreeding levels and hybrid introgression led to the recognition that new germ-plasm was required to reinvigorate the industry. As wild stocks of Nile Tilapia were still plentiful in northern Africa a stock improvement project adopted an integrated approach to developing a new strain with high productivity for the industry in Asia. The new

strain referred to as the 'Genetically Improved Farmed Tilapia (GIFT strain) was developed in the Philippines as a result of integrated applied research in the fields of: molecular population genetics, animal breeding and quantitative genetics to deliver a culture strain that grew >60% faster than other tilapia culture lines available there (Eknath *et al.*, 1993). Molecular population geneticists collaborating on this project characterised the genetic diversity in wild african populations and these data were used to undertake strategic collections of compatible, yet highly diverse, wild river strains that, after evaluation in culture as inbred lines, were later combined into a synthetic culture strain that was taken through multiple generations of family selection to improve growth rate. This strain was later disseminated widely in the Asia-Pacific region to reinvigorate the culture industry. Some recent reports have suggested however that the productivity of some GIFT culture stocks have already declined and the suggestion is that this may result from poor stock management leading to high inbreeding rates and hybrid introgression with local strains. Ongoing monitoring of genetic diversity levels in GIFT culture stocks is rare, but had this been practiced, could have alerted the industry to the problem before it became a major issue. Atlantic salmon and Channel catfish stock improvement programs have not made the same mistake and routinely monitor genetic diversity in both cultured and wild stocks.

CONCLUSIONS

Thus, questions about the utility of population genetics in aquaculture has, in my opinion, focussed on a very narrow aspect of the application of the science, i.e. direct production of improved strains. As outlined above, there are other important related issues with farmed aquatic species where applied molecular population genetics research has a significant role to play. In many cases, these are not issues that are highly relevant for improvement programs in terrestrial farmed animal species. Maintaining healthy levels of genetic diversity in cultured aquatic species over the long term constitutes a much greater problem than for equivalent terrestrial animal species and molecular population genetics when applied appropriately, provides powerful tools to address

this issue. Specifically, the approach can (1) allow effective documentation of wild genetic resources, (2) assist in better broodstock selection, (3) be used to monitor the impact of hatchery practices on genetic diversity levels in the hatchery and growout cohorts, (4) be used to develop a better understanding of the social and reproductive behaviour of target species and (5) to design better breeding systems and (6) can be used to monitor impacts of hatchery genes on wild populations. Hence, I am confident that applied molecular population genetics has an important role to play in the future development of aquaculture in the Asia-Pacific region and should be more closely integrated into breeding programs and quantitative studies of aquatic species targeted for stock improvement programs in the future.

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