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Molecular markers and their application in genetic diversity of penaeid shrimp

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Abstract Penaeidae is a family of shrimp, and it contains many species of economic importance, such as the tiger prawn (*Penaeus monodon*), white leg shrimp, Atlantic white shrimp and Indian prawn. Identification and population genotype structure of penaeid shrimp have been enhanced by molecular markers that can be classified into three types, namely allozyme, mitochondrial and nuclear markers. The widely used mitochondrial DNA markers are 12S rDNA, 16S rDNA, cytochrome b and control region. Random amplification of polymorphic DNA, amplified fragment length polymorphism, restriction fragment length polymorphism, single-stranded conformational polymorphism and microsatellites are the most commonly used nuclear markers for DNA fingerprinting. Molecular markers play a crucial role in penaeid shrimp to evaluate phenotypic and genetic variation, assess demographic bottleneck, study natural population structure, compare wild and hatchery populations, preserve genetic biodiversity, construct chromosome maps and detect whether genetic tag propagation–assisted rehabilitation programs are effective. Increase in the number of molecular markers, construction of high-density genetic maps and implementation of genomic resources (including genome sequencing) are expected to provide tools for the genetic improvement in these aquaculture species through marker-assisted selection. Molecular markers are versatile tools for the identification of populations with genetic crisis by comparing genetic diversities, which helps to establish management units within these threatened species.

Keywords Penaeid · Molecular marker · Taxonomy · RAPD · RFLP · Genetic diversity

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Introduction

Understanding the population genetic structure is an important component of successful and sustainable long-term management of fishery resources (Hillis et al. 1996). Genetic diversity can be defined as genetic variation between and within species, which is measured by determining the proportion of polymorphic loci across the genome or by determining the number of heterozygous individuals in a population and different genetic combinations in a gene pool. Genetic diversity is important for survival of a species and its adaptability to the environment. In contrast, a species that has little or no genetic diversity will produce offspring that are susceptible to diseases and have reduced biologic fitness and increased chances of species extinction. Higher-order taxonomic level is due to evolutionary forces such as non-random mating, small population, mutation, genetic drift recombination and natural selection. Genetic variation includes substitution, insertion, deletion, inversion and rearrangement of a DNA segment around a locus of interest. Genetic variations are heritable and are phenotypically and genetically recognizable. In penaeid population, detection of the genetic variation using different molecular markers is useful to geneticists in genetic improvement program of economically valuable species. Population variation can be detected by restriction digestion, electrophoresis and sequencing. While deciding the type of genetic marker to be used to characterize genetic diversity in a new study, a number of issues should be considered: (1) choice of marker to a large extent will depend on the research question(s) to be addressed—effective markers have to be chosen to address specific research questions; (2) relative costs, technical difficulty and necessary facilities/equipment have to be compromised—most powerful marker available has to be chosen within resource limitations, with the capacity to address the questions; and (3) data have to collected from different types of markers (e.g., combining data from nDNA and mtDNA markers).

The history of molecular genetics dates back to the early 1950s when F. Crick, J. Watson and M. Wilkins established the currently accepted model of DNA structure (the double helix). Since then, details of structure and function of DNA and genes have been clarified and started to be used in determining the genetic diversity. Methods for DNA cloning, sequencing and hybridization developed in the 1970s, and DNA amplification and automated sequencing, which were developed in the 1980s, led to the development of various classes of DNA markers. The classical molecular technique for studying genetic variation at codominant Mendelian-inherited loci is allozyme electrophoresis. The technique was developed in the 1960s and was dominating until the early 1990s. In the early 1980s, the first population genetic studies based on the analysis of mitochondrial DNA emerged (Avise et al. 1979). Later, with the advent of the PCR, a number of different techniques emerged, ranging from sequencing of the DNA of interest to methods analyzing length polymorphisms, such as microsatellites (Hansen 2003).

In genetics, a molecular marker is a fragment of DNA that is associated with a certain location within the genome. Molecular markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. Popular genetic markers in the aquaculture community include allozymes, mitochondrial DNA and nuclear markers such as RFLP, RAPD, AFLP, microsatellite, SNP and EST markers. Allozymes, as a genetic marker, are used in population genetics (Benzie et al. 1992; David et al. 2003; Nei 1978) and phylogenetic analysis in crayfish (Fetzner 1996; Chiesa 2009) and lobsters (Chu et al. 1990). Mitochondrial DNA, the extensively used genetic marker in shrimp species (Garcia-Machado et al. 2001; Klinbunga et al. 2001; McMillen-Jackson and Bert 2003) due to its favorable features such as non-recombination, maternal inheritance

and high mutation rate (Castro et al. 1998), has proved to be useful in several population genetic and phylogeographic studies. Application of DNA markers has allowed rapid progress in aquaculture investigations of genetic variability and inbreeding, parentage assignments, and species and strain identification and in the construction of high-resolution genetic linkage maps for aquaculture species (Liu and Cordes 2004). Various authors reported about the studies related to broodstock management including breeding programs, genetic mapping and analysis of population structure (Sbordoni et al. 1986; Bouchon et al. 1994; Tzeng and Yeh 1999, 2002; Hetzel et al. 2000; Sugaya et al. 2002; Li et al. 2003; Jerry et al. 2004). The last decade has seen a renewed interest in coding genes for studying the association of their variability with economically important traits. In this respect, comparative genomics can greatly accelerate the identification of effective markers, because many genes are very conservative, allowing the transmission of information between species, with reduction in time and costs.

Molecular and genetic manipulation technologies have been widely used to improve several species (Dall et al. 1990; Sagi and Cohen 1990; Xiang et al. 1992), with polymerase chain reaction (PCR) technology. Population genetics of many penaeid shrimps have been well studied, since the knowledge of the genetic structure of fisheries stocks is crucial to shape policies that will ensure sustainable stock viability. 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. Attempts to integrate available molecular tools are limited by the lack of information and investment on shrimp in the process. Much 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 (Benzie 2010). This review article is intended to provide a systematic introduction of molecular marker technologies and their applications in genetic diversity of penaeid aquaculture.

Molecular markers

Allozymes

The term ‘allozyme’ refers to products of different allelic forms of an enzyme-coding gene. These products are strands of amino acids called polypeptides. Among 20 common amino acids that make up polypeptide chains, five have weak electric charges (lysine, arginine and histidine are positively charged; aspartic acid and glutamic acid are negatively charged). Thus, polypeptide chains comprising different numbers of these amino acids have different net electrical charges. The allozyme molecule is made of one or more than one type of polypeptide chains. As crude extracts of enzymes are placed in an electric field, allozyme molecules will be separated according to their net charge. Allozyme electrophoresis involves the separation of products from isozyme alleles on the basis of differential migration due to varying surface charge when subjected to an electric current. Thus, different alleles are detected based on the mobility differences of their products at the end of the run, which are visualized via specific histochemical staining procedures (Richardson et al. 1986). Studies on allozyme electrophoresis commenced in the late 1960s and early 1970s, and now a huge data set is available. There are four most commonly used methods of allozyme electrophoresis, depending on the type of medium used: starch, polyacrylamide, agarose and cellulose acetate. Allozymes have several advantages: they are

relatively inexpensive and quick and they provide a sensitive technique for screening genetic diversity, codominant nature and availability of protocols common for a wide range of organisms. Differences in the presence/absence and relative frequencies of alleles are used to quantify genetic variation and distinguish between genetic units at the levels of populations, species and higher taxonomic designations. Limitations of using allozymes are as follows: the limited number of available allozyme loci, which precludes their use in large-scale genome mapping; heterozygote deficiencies due to null (enzymatically inactive) alleles; inability to read genotypes from small quantities of tissue; requirement of fresh tissue; tissue-specific expression exhibited by many loci; and the technique detects only a portion (usually <25 %) of the actual genetic variation because not all nucleotide changes lead to amino acid substitutions. Additionally, not all amino acid substitutions result in electrophoretically detectable mobility differences.

Mitochondrial DNA

Mitochondrial DNA (mtDNA) differs significantly from nuclear DNA in structure and mode of inheritance. mtDNA is a circular molecule that undergoes no recombination and is maternally inherited. The molecule (generally 16–20 kb in size) is made up of 13 protein-coding genes, 2 ribosomal RNA genes (rRNA), 22 transfer RNA genes (tRNA) and a section generally known as the D-loop or control region (which is non-coding but is involved in the replication of the molecule). Unlike the nuclear genome, the mitochondrial genome contains very little non-coding DNA. Several characteristics of mtDNA make it a good choice of molecular marker for population studies. Firstly, its maternal inheritance and haploid nature show that populations will exist at one-fourth of the effective population size (*Ne*) as that seen with nuclear markers. This characteristic will amplify the effects of drift (causing populations to differentiate), so mtDNA is therefore sensitive for detecting population structure. Secondly, the lack of recombination means that mtDNA lineages will evolve without the history of descent becoming jumbled over time as on homologous chromosomes. This allows us to differentiate between historical and contemporary processes that may have influenced or determined the observed population genetic structure. Thirdly, mtDNA is generally considered selectively neutral (although this has been challenged recently). Therefore, the effects of selection can largely be removed as a confounding factor when interpreting the data. The limitation is due to non-Mendelian mode of inheritance, and the mtDNA molecule is considered as a single locus; because mtDNA is maternally inherited, the phylogenies and population structures derived from mtDNA data may not reflect complete picture of the nuclear genome if migration is gender-biased.

Nuclear DNA marker

Restriction fragment length polymorphism (RFLP)

RFLPs were the first DNA markers to be used by population biologists (Parker et al. 1998). The technique involves cutting a DNA strand at specific nucleotide sequences using a restriction endonuclease and thereby producing a pool of DNA fragments of different sizes. RFLP variation can be visualized directly by staining with ethidium bromide following electrophoresis of the DNA in an agarose gel. This can be done for small molecules, such as the entire mtDNA, which produce a manageable number of fragments with many restriction enzymes. The most appropriate method of analysis involves restriction sites,

whereby actual sites are mapped to specific positions on the strand of DNA of interest. Scoring is based on the loss or gain of a site, and this gives an accurate resolution of relationships. An alternative method is fragment analysis, which scores the different fragments as either present or absent. However, this method assumes that fragments of similar length on a gel are homologous. Unfortunately, this assumption can be misleading since multiple fragments may make up a single band on a gel. In addition, different cleavage sites may produce similar banding patterns, thus giving erroneous relationship between samples (Fetzner and Crandall 2001). A more efficient approach that is now used relatively commonly is to amplify the DNA region of interest and then conduct restriction analysis on the amplified fragment. PCR products are treated with restriction enzymes, and the fragments are separated on an agarose gel and visualized by ethidium bromide staining to identify RFLP profiles. An advantage of this approach is that the PCR fragment can first be sequenced from a number of individuals in the first instance, to allow the detection of polymorphic restriction sites. mtDNA is most commonly used for this method of genetic analysis as it has been proven to allow easy detection of genetic differences at population levels.

Random amplification of polymorphic DNA (RAPD)

RAPD is a random amplification of anonymous loci by PCR. The method is simple, rapid and cheap, has high polymorphism, requires only a small amount of DNA and allows creation of genomic markers from species of which little information is known about the target sequences to be amplified. RAPD markers are produced by PCR using short oligonucleotide primers of random sequences. Different RAPD patterns arise when genomic regions vary according to the presence/absence of complementary primer annealing sites. The primers are typically 10 bp long (Williams et al. 1990), and no specific knowledge of a particular DNA sequence is required. Allelic variation usually consists of the presence/absence of particular amplification products, which can be separated on agarose gels stained with ethidium bromide. First of all, RAPD by PCR has the following major requirements: a small amount of genomic DNA, one or more oligonucleotide primers (usually about 10 base pair in length), free nucleotides and polymerase with a suitable reaction buffer. This methodology has some disadvantages, including difficulty in reproducing results, subjective determination of whether a given band is present or not and difficulty in analysis due to the large number of products. This is because RAPDs are not sensitive to any but large-scale length mutations. Therefore, variation might be underestimated as the technique is based on the PCR amplification of discrete regions of genome. These problems have limited the application of RAPDs in aquaculture studies. RAPDs have gained considerable attention particularly in population genetics, species and subspecies identification, phylogenetics, linkage group identification, chromosome and genome mapping, analysis of interspecific gene flow and hybrid speciation, and analysis of mixed genome samples breeding analysis and as a potential source for single-locus genetic fingerprints (Brown and Epifanio 2003).

Amplified fragment length polymorphism

AFLP was introduced by Vos et al. (1995). The AFLP protocol involves the following steps: DNA digestion with two different restriction enzymes (typically EcoR I and Mse I), ligation of double-stranded adapters to the ends of the restriction fragments, optional DNA pre-amplification of ligated product directed by primers complementary to adapter and

restriction site sequences, DNA amplification of subsets of restriction fragments using selective AFLP primers and labeling of amplified products, separation of fragments via electrophoresis, and scoring fragments as either presence or absence among samples. The key feature of AFLP is the capacity for simultaneously screening many different DNA regions distributed randomly throughout the genome. AFLP combines the strengths of RFLP and RAPD markers and overcomes their problems. The approach is PCR-based and requires no probe or previous sequence information as needed by RFLP. It is reliable because of high stringent PCR in contrast to RAPD's problem of low reproducibility. The major advantage of AFLPs is that a large number of polymorphisms can be scored in a single polyacrylamide gel without the necessity for any prior research and development. AFLP seems to be much more efficient than the microsatellite loci in discriminating the source of an individual among putative populations. Similar to RAPD, AFLP analysis allows screening of many loci within the genome in a relatively short time and in an inexpensive way. The weakness is that they are dominant markers; thus, on average, half of them are useful for a given backcross reference family. The methodology is also difficult to analyze due to the large number of unrelated fragments that are visible (on the gel) along with the polymorphic fragments (as with RAPDs).

Microsatellites

Currently, microsatellites (or simple sequence repeats (SSRs) or variable number tandem repeats (VNTRs)) are the most favoured nDNA marker in genetic diversity studies as they usually show very high levels of individual and population variation. SSRs are either simple (e.g., TGTGTGTGTG) or complex (e.g., GAA (GA) 17 GAA) tandem repeats of short DNA sequences that are found at regular intervals right across the genome of most eukaryote species. The nuclear genome of eukaryotes contains segments of DNA that are repeated tens or even hundreds to thousands of time (O'Reilly and Wright 1995). These repeated sequences are the most important class of repetitive DNA. They repeat in tandem and vary in number at different loci and different individuals dispersed throughout the genome. Microsatellite loci can be identified by screening genomic libraries with probes made up of tandem repeated oligonucleotides and then sequenced to identify conserved flanking regions for primer design. Loci identified in this way are analyzed by amplifying the target region using PCR, followed by electrophoresis through a polyacrylamide gel to allow the resolution of alleles that may differ in size by as few as two base pairs. The microsatellites have been extremely useful in fish and crustacean population studies and are quickly becoming the marker of choice for a variety of applications. High polymorphism and the relative ease of scoring are the two major features that make microsatellites of large interest for many genetic studies. The major drawback of microsatellites is that they need to be isolated de novo from species that are being examined for the first time. High-output microsatellite library screening requires an automatic sequencer, which is available only in a few laboratories in Asian countries. However, in the case that primers used for microsatellites have been developed for different species, caution should be exercised. Ideally, target products should be sequenced to verify that they are 'real' microsatellites. It is also common in the region that the size of gels used to screen variation is often too small and much of the 'real' variation is not detected, leading to potential problems with Hardy–Weinberg equilibrium conformation. Laboratories should therefore consider applying a more rigorous approach to silver-staining microsatellite analysis or use less technically demanding nuclear markers such as allozymes.

Single-nucleotide polymorphisms (SNPs)

The difficulty to fully automate microsatellite genotyping has revived interest in a new type of markers. Single-nucleotide polymorphisms are polymorphisms due to single-nucleotide substitutions (transitions > transversions) or single-nucleotide insertions/deletions. SNP is a variant of single-copy nDNA polymorphism based on the detection of individual nucleotide. These variants can be detected using PCR, microchip arrays and fluorescence technology. Major applications of SNPs in fisheries are in genomic studies and are used as diagnostic markers for diseases. Since they are the main part of many gene chips, they are considered as next-generation markers in fisheries. However, they might provide marginal additional, or even less, utility in some applications (e.g., relatedness).

Expressed sequence tags

Expressed sequence tags (ESTs) are generated by single-pass sequencing of complementary DNA clones obtained by reverse transcription of messenger RNA (Putney et al. 1983). High-throughput sequencing generates information on thousands of ESTs, which can be compared with other DNA or protein sequences available in public databases. At the same time, the new sequences are made accessible in various databases, increasing the growing information on gene expression. As ESTs are the direct product of gene expression, their analysis leads directly to the description of the transcriptome, which is not the case with whole-genome sequencing projects. ESTs can be mapped to specific chromosome locations using physical mapping techniques, such as radiation hybrid mapping, HAPPY mapping and FISH. Alternatively, if the genome of the organism that originated the EST has been sequenced, one can align the EST sequence to that genome using a computer. In this respect, ESTs have become a tool to refine the predicted transcripts for those genes, which leads to the prediction of their protein products and ultimately their function. Moreover, the situation in which those ESTs are obtained gives information on the conditions in which the corresponding gene is acting. ESTs contain enough information to permit the design of precise probes for DNA microarrays that then can be used to determine the gene expression.

Amenability to automation

Automated techniques are described as a method of streamlining the enzymatic reaction involved in the amplification of nucleic acids and the use of robots to carry out the process, which is highly preferred because of the potential for high sample throughput. Although considerable financial investment is still required, automation may be cost-effective when techniques are applied on a routine basis. Outsourcing of data generation may also be an alternative strategy. Nearly all PCR-based techniques are amenable to a certain degree of automation.

Multiplex PCR

Multiplex PCR system can detect multiple target sequences using simultaneous amplification profiling in GM crops such as soybean, maize and canola (James et al. 2003). Uniform product abundance is an important feature of any multiplex amplification method, especially for highly paralleled SSR genotyping and the preparation of samples for SNP detection assays. Multiplex PCR is more sensitive, fast, easy to perform and beneficial with

limited sample, detects all organisms regardless of physiological state, obtains more information per unit time, economize on reagents, enzyme, buffers and labor, streamlines data analysis, uses standardized protocols to prepare PCR fragments for DNA fragment analysis and has a high tolerance to variation in the concentration and quality of DNA samples. Multiple-tube approach has been widely adopted by researchers to guard against allelic dropout (misidentification of loci as homozygous due to the chance amplification of only one of the two heterozygous alleles) and false alleles (mistyping due to random contamination, PCR slippage artefacts or other sources) (Taberlet et al. 1996, 1999; Broquet and Petit 2004). The multiplex-ready PCR technology provides several enabling advances in marker genotyping that substantially reduce assay costs, increase marker throughput and facilitate automation. Multiplex amplification with fluorescence detection enables high power of discrimination in a single test. Multiple loci were queried in a single PCR using the multiplex PCR kit and pooled primer sets that produced amplicons of length 100, 150, 200, 250, 300, 400, 500, 600, 700 and 850 bp, corresponding to up to 10 different genomic loci. In each 20 µl PCR, 1 µl of a 1-in-100 dilution in water of a saturated clonal culture (i.e., produced from a colony one wishes to assay) generated the best MASC-PCR specificity (Carr et al. 2012). This technique has applications in pathogen identification, high-throughput SNP genotyping, mutation analysis, gene deletion analysis, template quantification, linkage analysis, RNA detection and forensic studies.

Application

Genetic diversity in wild penaeid shrimp

Allozyme marker has been the most commonly used molecular marker for the study of penaeid populations (reviewed in Benzie 2000). Allozyme marker (Gusmao et al. 2000) codes for 14 loci for *F. subtilis*, *F. paulensis*, *F. brasiliensis* and *F. schmitti* collected from eight localities along Brazilian coast (>4,000 km). Two diagnostic loci, adenylate kinase (Ak) and phosphoglucornic dehydrogenase (Pgd), are there in two sympatric populations of two morphotypes of *Penaeus subtilis* from Fortaleza, which indicate that two morphotypes with non-exchanging genes are stable even over a considerable geographic distance (2,700 km), confirming that as separate species. Even though there is allozyme similarity between *P. subtilis* MII and *P. paulensis* ($I = 0.994$–0.981), no diagnostic loci were found between them; thus, allozyme study failed to support the statement of conspecificity between them proposed by D'Incao et al. (1998). The samples were collected from south of Fortaleza, the morphotype II was found from Fortaleza (Ceara) to Cabo Frio (Rio de Janeiro), whereas no *F. subtilis* MI was detected. The accepted distribution of *L. subtilis* from Caribbean south to Rio de Janerio (Perez Farfante 1969) is the result of sum of the distribution of morphotypes I and II species, with narrow overlapping in Northeast Brazil. The phylogenetic relationship of these species is paraphyly that *F. brasiliensis* separated from groups formed by *F. subtilis*, *F. paulensis* and *L. schimitti*. De la Rosa-Velez et al. (2000) studied the commercial penaeid shrimp of *P. californiensis* and *P. stylirostris* from Gulf of California to assess 32 loci of allozyme—16 from enzyme, remaining from muscular protein. Protein polymorphism showed that genetic variability parameter is 0.125 for *P. californiensis* in three populations, whereas *P. styliristris* showed north–south cline-like pattern in its genetic variability parameter from 0.156 to 0.312. The population structure is more for *P. stylirostris* because of the more stable ocean condition, the life cycle of *P. californiensis* is completed, whereas *P. stylirostris* lives in coastal lagoon

(post-larval stage) and matures only in the ocean. In coastal lagoon, the latitudinal variability of hydrological, ecological and productive conditions has been recorded along eastern coast of California. The upper zone of lagoon is arid with low biodiversity, so production has been restricted to south Gulf, which is of high biodiversity with complementary sources of organic production with detritus chain. Thus, the complexity of ecological web increases from north to south of coastal zone of California. The gradual increment in heterozygosity in the population of *P. stylirostris* may be related to those ecological features. Both species have good population heterogeneity perhaps due to the existence of private alleles in polymorphic loci and the most frequent alternation of alleles in the subpopulation of same species at different loci. In addition, the upper Gulf region was separated from south submarine by Tiburon and Angel de la Guarda Islands, which could act as barriers to gene flow. In some populations of both species, three loci (Aph-1, Est-2 and Pgm) deviate from Hardy–Weinberg equilibrium according to chi-square fitness, and the possible deficiencies in heterogeneity are inbreeding and selection against heterozygote. In a population of *F. notaialis* in Cuba, an investigation into a 25-locus sample of shrimp from 7 localities in Ana Maria Gulf revealed 9 polymorphic strong population structures with significant F_{ST} in all pairwise comparisons, the level of differentiation being concordant with geographic localities and the hydrographic regime (Garcia-Machado et al. 2001).

Arena et al. (2003) reported the allozyme analysis of genetic relationship between *L. stiferus* (Mexico), *L. schmitti* (Cuba), wild *L. setiferus* (Rio Tuxpan outlet) and *L. shmitti* (Rio Cauto outlet). These species differ greatly in the frequency of the most common allele in eight loci. Loci pt-2 and Est-2 have different alleles in higher frequencies for each species. The frequencies of all enzymes showed significant differences between species but similarity between populations. Tuxpan populations deviate from Hardy–Weinberg expectation that loci Est-3, Amy and Phos-1 are the possible subdivisions of population by combination of ecological elements. Genetic diversity is low among population of same species but high in different species. The expected heterozygosity is 0.114 for *L. setiferus* and 0.0083 for *L. schmitti*; the lowest value of distance is for *L. schmitti* population, and differentiation is high within population than that between populations. A 35 % difference at loci was analyzed between two species. Thus, closely related populations of *L. setiferus* and *L. schmitti* are cultured in Mexico and Cuba, permitting to design strategies to preserve both the local gene pool and the genetic variability. Investigation into the same species and location of a previous study along Brazilian coastline revealed that no population heterogeneity was detected in *F. brasiliensis* and *L. schmitti* ($F_{ST} = 0.011$ and 0.024) population along the study area (Gusmao et al. 2005). In contrast, F_{ST} values for *Farfantepenaeus* sp. ($F_{ST} = 0.032$) and *F. paulensis* ($F_{ST} = 0.045$) indicate that the populations of these two species are genetically structured, comprising different fishery stocks as reported in the year 2000. The mean F_{ST} observed for *L. schmitti* ($F_{ST} = 0.0234$) within the range of *F. notialis* population ($F_{ST} = 0.021$–0.038), which is consistent with homogeneity, has been reported for many other penaeid shrimps (reviewed in Benzie 2000). The largest genetic differences in *F. paulensis* were found between species from Lagoa dos Patos (south) and two populations from Southeast Brazil. In *Farfantepenaeus* sp., significant differences were observed between populations from Recife, Fortaleza ($\chi^2 = 27.49$; $p < 0.05$) and Ilheus ($\chi^2 = 30.57$; $p < 0.01$). Similar levels of population genetic structuring have also been observed for Californian populations of *L. stylirostris* (Ramos-Paredes and Grijalva-Chon 2003) and *F. californiensis* (De la Rosa-Velez et al. 2000). Mean heterozygosity values observed for these species ($H = 0.02$–0.10) were similar to those of earlier surveys ($H = 0.006$–0.175) (Benzie 2000; Garcia-Machado et al. 2001; Espinosa et al. 2002; Ramos-Paredes and Grijalva-Chon 2003). The *Mpi* locus

showed a relatively high variability among *Farfantepenaeus* species, which could be due to the tertiary structure and hitherto unknown balanced selection on that locus. Significant deviations from Hardy–Weinberg expectations of the *Pgm-1* locus in two populations of *F. paulensis* indicate population mixing, so that populations separated by 1,000 km can be genetically more similar than those separated by very short distances. The level of worldwide genetic population varies between different species of shrimp, and the patterns of differentiation observed are more likely to reflect the present-day dispersal, rather than biogeographic patterns as far as the Brazilian shrimp populations is concerned. In spite of their different levels of population structuring, species studied here have quite similar distribution ranges. *F. brasiliensis* and *L. schmitti* population do not present any significant differentiation from the south of Brazil to the Caribbean and have wide overlapping zones with the distributions of *F. paulensis*, in the south of Brazil, and of *Farfantepenaeus* sp., in Southeast and Northeast Brazil. Thus, the incongruence found between the population structure patterns cannot be attributed to common biogeographic boundaries. Moreover, the observed levels of differentiation among populations of *F. paulensis* and *Farfantepenaeus* sp. do not seem to indicate a direct correlation with geographic distances. Instead of biogeographic factors, the observed differences in population structure between the four species studied seem to reflect the differences in their biology. Among Brazilian shrimp species, the periods of reproduction, estuary post-larval penetration and subadult emigration vary depending on the species, and even among populations of the same species in different geographic regions, differences in environmental preferences in relation to water temperature, salinity, sediment type and bathymetry were observed between species. For example, *F. paulensis* is found in cold waters, it usually spawns below 50 m depth, and its migration is more related to depth than to latitude. *F. brasiliensis* and *L. schmitti*, on the other hand, prefer warmer waters, so that migration of *F. brasiliensis* is more related to latitude than to depth. As evident in studying two breeding populations in different geographic areas, populations of *F. paulensis* from Rio de Janeiro and Santos (Southeast Brazil) are significantly different from those from Lagoa dos Patos (south). Hence, genetic variation and genotype distribution may be related to life history, biology, historical events and biogeographic factors.

Voloch and Sole-Cava (2005) reported that 8 enzymes coded for ten allozyme loci on *Xiphopenaeus kroyeri* along the Southeastern Brazilian coast clearly indicated that the São Paulo population is highly structured ($F_{ST} = 0.223$) and independent stock from those of Rio de Janeiro and Espirito Santo. High unbiased genetic distance (D) values are found between the population from São Paulo and those from Rio de Janeiro and Espirito Santo ($D_{SP\text{-}RJ} = 0.17$; $D_{SP\text{-}ES} = 0.19$), whereas populations from Rio de Janeiro and Espírito Santo are very similar to each other ($D_{RJ\text{-}ES} = 0.00$), which indicates that a barrier to gene flow exists between the São Paulo and Rio de Janeiro populations. Two aspects that might influence the population dynamics of *X. kroyeri* are their distribution exclusively in shallow waters and their independence from estuaries to complete their life cycle. This indicates that other environmental factors could be the cause for the observed structure; particularly, temperature variation may be influencing the population dynamics of *X. kroyeri*. Robainas-Barcia et al. (2005) reported the temporal variation of allozymes in a shrimp species inhabiting Cuba, which proves that the genetic structure of this species could significantly change in time. Their study involved four populations of *Farfantepenaeus notialis* sampled in a period of 8 years. The significant statistics obtained from partitions observed in 1995 were not detected in 2003 (as suggested by AMOVA and F_{ST}), whereas temporal genetic differentiation and heterozygosity became highly significant. The results strongly suggest that the effect of migration could be the cause for the loss of

genetic structure of *F. notialis* in 2003. A study of *F. penicillatus* showed high level of genetic diversity in each population, of which 2.09 % is interpopulation genetic diversity and remaining 97 % is intrapopulation genetic diversity (Zhao et al. 2009). This result shows that genetic structure is very similar among nine populations.

Since mtDNA is haploid and transmitted maternally, the effective population size estimated from mtDNA is generally smaller than those of nuclear markers such as allozymes and nuclear DNA. For mtDNA, sensitivity to inbreeding and bottleneck effects are relatively higher than those of nuclear DNA markers. Therefore, analysis of genetic diversity by sequencing of mtDNA genes is a more direct and sensitive method for evaluating the genetic population structure of wild penaeids accurately. In Gulf of California, Mexico, Aubert and Ligheter (2000) investigated *P. stylirostris* by RAPD. Samples with previously identified distinct morphological characteristics were collected from 6 sites. A total of 24 RAPD loci from 78 specimens were subjected to quantification and comparison between stock genetic differences. The factors for the formation of two distinct population structures are as follows: evolutionary forces in isolated populations, geographic barrier, physiological and morphological adaption and finally reproductive isolation, which all lead to the evolution of new species.

Using mtDNA with RFLP, Benzie et al. (2002) reported in a population of *P. monodon* in Indo-Pacific region that no haplotype was in common for five populations studied: three from Southeast Asia, one from southeast Africa and one from Australia. The dominant haplotype is different in the Australian and Southeast Asian populations. Genetic diversity is highest in Indonesian population (averaged 0.05), less in the Philippine and Australian populations (averaged 0.01) and markedly less in southeast African and West Australian populations (averaged 0.003). The high diversity of the Southeast Asian populations resulted in the occurrence of a set of haplotypes derived from the southeast African haplotypes. It is clear that these genetic variants evolved from Indian Ocean and later migrated into the Indo-West Pacific region. Low genetic variation in the geographically marginal populations in southeast Africa and West Australia is considered to be the result of bottlenecks, but mismatch distributions suggest that large population sizes have been maintained in Indonesian populations for long periods. This finding evidences that the Indo-West Pacific region is a site of accumulation of genetic diversity rather than a site of origin of genetic diversity. Cui et al. (2007) analyzed mtDNA variation in *Penaeus kerathurus* prawns collected from seven locations along a transect across the Siculo–Tunisian region in order to verify whether any population structure exists over a limited geographic scale and to delineate the putative transition zone with sufficient accuracy. Partial DNA sequences of COI and 16S genes were analyzed. In contrast to the highly conservative 16S gene, the COI sequences exhibited sufficient diversity for population analysis. The COI gene revealed low levels of haplotype and nucleotide diversities. The size of the annual landings of this commercial species suggests large population sizes. Hence, the low genetic diversity detected in this study could indicate a possible reduction in effective population sizes in the past.

Klinbunga et al. (2001) investigated *Penaeus monodon* collected from five areas, Chumphon and Trat (Gulf of Thailand), and Phangnga, Satun and Trang (Andaman Sea). They used RAPD and mtDNA (16S ribosomal DNA and an intergenic COI-COII) polymorphism. In mtDNA analysis, 37 mtDNA composite haplotypes, and high haplotype diversity (0.855) and nucleotide diversity (3.328 %) of Thai *P. monodon* were observed. Population differentiation of *P. monodon* between the Andaman Sea and Gulf of Thailand was clearly illustrated by both techniques ($p < 0.0001$). The results of population differentiation are genetically significant between Chumphon and Trat while using RAPD,

whereas these results did not show differentiation between these samples while using mtDNA polymorphism. From these findings, they concluded that under the presumption of selective neutrality of this mitochondrial marker, biased female gene flow between Trat and Chumphon *P. monodon* may exist and be responsible for an anomalous differentiation pattern.

Khamnamtong et al. (2009) reported on a population of *P. monodon* in Thailand waters (Satun, Trang, Phangnaga and Raong in Andaman sea, and Chumphon and Trat in the Gulf of Thailand) examined by mitochondrial cytochrome oxidase subunit I (COI) polymorphism. They suggested the presence of many specific unique mitotypes with low frequency in each geographic sample as reported by PCR–RFLP of the entire mtDNA (Klinbunga et al. 1999) and PCR–RFLP of 16S rDNA and COI–COII (Klinbunga et al. 2001). The average nucleotide divergence between a pair of COI mitotypes across all specimens is 6.604 %; thus, genetic diversity is high. Ranong sample had high haplodiversity (0.927) and lowest nucleotide diversity (2.9360). This shows that small number of samples is not enough for a large geographic location. Since intraspecific population structure exists in Thai *P. monodon*, six samples can be grouped into three categories: Trat (A); Chumphon (B); and Satun, Trang, Phangnga and Ranong (C). High genetic divergence in Thai *P. monodon* indicates that each genetic population is a separate management unit because it displays unique demographic and dynamic properties for genetic improvement in the commercially important traits through selective breeding program.

Benzie (2000) reviewed penaeid prawns and concluded that estimated variability from DNA-based markers showed high level of diversity in natural population than that inferred from allozyme; however, DNA-based markers tended to confirm observation based on allozyme data. Niamaimandi et al.'s (2010)study was the first RAPD study of the population of *Peaneus semisulcatus* in Busher (Persian Gulf), which were collected from Halailechan and Daylam, using 9 primers. Percentage of polymorphism is 14.8 %, and this indicated that populations around Bushehr are not well structured, which is consistent with a previous PCR–RFLP analysis by Rezvani et al. (2001) and Rezvani (2002).

Random amplification of polymorphic DNA is a simple and easy molecular method to estimate genetic diversity in penaeid shrimp, fish and shell fish (Zhuang et al. 2001a, b; Lakra et al. 2010). Mishra et al. (2009) collected 105 samples of *Metapenaeus dobsoni* from three locations: Mumbai in Maharashtra, Tuticorin in Tamil Nadu and Kochi in Kerala, and they used 5 random primers for PCR amplification. The polymorphic loci varied from 22.3 to 40.9 % in the three populations. The Maharashtra population had high diversity value (40.9 %) and Kerala population, the lowest (22.3 %). Zhuang et al. (2001b) detected low polymorphism (20–33.3 %) in four populations of *P. chinensis*. In dendogram, *M. dobsoni* populations from the west coast of India—Kerala and Maharashtra—clustered together, but Tamil Nadu formed a separate group as east coast of India. The authors recommended that even though RAPD technique is very advantageous as a preliminary tool for studying the population structure of penaeids, it could be accompanied with microsatellite analysis.

Rezvani Gilkolaei et al. (2011) analyzed *Fenneropenaeus indicus* reared in the same situation for 4 months and divided the sample into 3 groups as high, medium and low growth based on weight and length using RAPD tool. Among 21 loci studied, 12 produced polymorphic bands. The highest genetic distance was observed (0.457) between low-growth and medium-growth group and the lowest (0.091) between high-growth and medium-growth groups, which indicates that RAPD maker is likely to assess enough genetic variation for the establishment of marker-assisted selective breeding program in penaeids. Garcia and Benzie (1995) reported 6–7 % polymorphism in *P. monodon* and

suggested that RAPD approaches will be useful in providing marker for penaeid breeding. While considering mean weight of F1(16.25 ± 1.5 g), parental generation mean weight (15 ± 1.2) and mean weight of parent (31.6 g), response to selection and heritability of growth are estimated to be 1.2 ± 0.2 and 0.07 ± 0.01, respectively; this shows that the total is attributable to the differences in breeding value. Recently, studies on genetics indicated a very good heritability of productive traits for penaeid shrimp, and this could open a good outlook to detect related genes for marker-assisted selections in future selective breeding programs of shrimp (Hoa 2009). Estimates of heritability of harvest weight have been reported: 0.16–0.31 in *M. japonicus* (Hetzel et al. 2000); 1.32 ± 0.18 in *L. vannamei* (Perez-Rostro et al. 1999); 0.24 ± 0.05 (full-sib family) and 0.17 ± 0.04 (half-sib family) in *L. vannamei* (Gitterle et al. 2005b); and 0.24–0.35 (univariate animal model) and 0.37–0.45 (multivariate animal model) in *L. vannamei* (Castillo-Juarez et al. 2007). In *L. vanna*mei, heritability of growth, Taura syndrome virus (TSV) resistance and tail percent were estimated to be 1 ± 0.12, 0.28 ± 0.12 and 0.15 ± 0.12, respectively (Argue et al. 2002); heritability value for body length was estimated to be 0.22 at 119 days of age (Perez-Rostro and Ibarra 2003) and 0.43 at 25 days of age (Campos et al. 2006); heritability of resistance to disease (Gitterle et al. 2005a; Ibarra et al. 2007) and reproductive traits (Arcos et al. 2004, 2005; Macbeth et al. 2007) has also been evaluated. Data from different commercial hatcheries with different abilities confirm the role of genetic control in size and growth of Penaeidae (Chow and Sandifer 1991). The range of heritability (0–1) in the larval stage of *L. vannamei* and *L. stirostris* shows the effects of environmental factors on growth (Lester and Lauser 1990). Argue et al. (2002) studied heritability of sex ratio in *L. vannamei*, which was not significantly different from zero. This differs from the results reported in turtles and fish, which exhibit significant heritability estimates for sex ratio (Lester et al. 1989); hence, instead of selective breeding, more females may possibly be produced by manipulating the androgenic gland or exposing shrimp to exogenous hormones (Sagi and Cohen 1990; Moss et al. 2002). In this study, heritability of weight for *F. indicus* was estimated to be 0.07, which is lower than those reported for other prawns, which could be explained by lack of genetic care during the domestication period. Estimation of response to selection in *M. japonicus* (Hetzel et al. 2000), *L. stylirostris* (Goyard et al. 2002) and *L. vannamei* (Argue et al., 2002) was, on average, 4–18 %, 10.7 % and 25 %, respectively, after one generation. In this study, response to selection was estimated to be 1.2 ± 0.2 (8 %) in one generation, which is lower than those observed in other marine species. Low rates of response observed in this study are presumably because of the relatively low selection intensity applied. Nevertheless, this study has demonstrated that in *F. indicus*, growth will respond to selection. It is expected that response to selection is valid only in the first generation, but it has been shown in the experiments that response with little change has been maintained during several generations (up to 5, 10 or even more generations). Over the longer term, phenotypic variation and heritability may decrease, resulting in lower rates of genetic change.

Maggioni et al. (2003) analyzed the population structure of *L. schmitti* occurring only in Brazil and reported that six microsatellite loci were observed across eight geographic locations. Pairwise estimates and AMOVA revealed a significant discontinuity around a major biogeographic boundary near Cabo Frio. This result shows that the population structure in penaeid shrimp may be influenced by environmental factors that exist across relatively small geographic scales and that associated with coastal waters. These may be related to large-scale biogeographic barriers recognized in other taxa, which have been previously suggested as influencing population structure in penaeid shrimp by Benzie (2000). In another study, Chiang et al. (2012) developed a microsatellite array for

Metapenaeus barbata collected from Taiwan and China. Eight dinucleotide SSR units were isolated, the number of alleles, ranging from 2 to 4, as well as observed and expected heterozygosities in populations, ranging from 0.048 to 0.538, and 0.048 and 0.654, respectively. There was no deviation from Hardy–Weinberg expectation at either locus and significant disequilibrium between any pair of loci. The locus-wise FIS for each population was non-significant after Bonferroni correction, indicating heterogeneity deficiency, except in one locus (MIMBO3) in Fuijan population. From these results, the microsatellite can be used as an effective marker for population studies of penaeid shrimp.

United States Marine Shrimp Farming Program (USMSFP) constructed a preliminary low-density linkage map (ShrimpMap) for specific pathogen-free shrimp *L. vannamei* based solely on SSRs or microsatellite genetic markers, to facilitate integration of linkage and physical maps, assist conservation and evolutionary genomics in penaeids and search for genes associated with fitness traits in wild and cultured shrimp. The map includes the first EST-SSR markers mapped for *L. vannamei*. Human ESTs also amplified in shrimp DNA, and two of them were useful for linkage analysis. Moreover, the mapped EST-SSRs provide valuable comparative genomic links not only between *L. vannamei* and other penaeid species but also between shrimp and other invertebrate and vertebrate genomes (Alcivar-Warren et al. 2007). In China, genetic linkage map for *Penaeus* (*Fenneropaeneus*) *chinensis* was constructed based on 354 markers, of which 300 are AFLP markers, 42 SSR markers and 12 RAPD markers. Forty-seven linkage groups were identified; the total map length was 4,580.5 cM, with an average spacing of 11.3 cM, covering 75.8 % of the estimated genome size. Emphasis has to be given for genetic improvement to identify QTL (quantitative trait linkage) connected with economically important traits and to promote the progress of genetic breeding programs in *F. chinensis* (Bo et al. 2009). As compared to the previous *F. chinensis* map (Li et al. 2006), the genetic linkage map presented in this study is improved, and the genome coverage increased from 69.6 to 75.8 %. Although the amp provides good coverage, 11 gaps (adjacent marker interval >30.0 cM) on LG4, 5, 13, 19, 24, 26, 36, 39 and 41 remain. The discrepancy between the haploid number and the linkage group number is an indication of large gaps and relatively low marker density.

Genetic diversity in cultured penaeids

Wuthisuthimethavee et al. (2005) reported a microsatellite-based linkage map construction in *P. monodon*, using 57 SSLP, 1 EST, 1 SCAR markers and 76 individuals of an F1 intercross family. The segregation marker is scored and analyzed with the Join-Map 2.0 program, and a total of 50 marker loci that are confirmed to follow Mendelian segregation ratio (c2 test at the a = 0.01 level) were grouped with an LOD score of 5.0. Results showed 9 linkage groups with 27 loci and 23 unlinked loci. This linkage map covered a total genetic distance of 103.6 cM. This genetic linkage map mapped more markers on the *P. monodon* genome to reference families. de Francisco and Galetti (2005) established the genetic relationships between five broodstocks of the white marine shrimp *L. vannamei* (Penaeidae) based on sequencing of the mtDNA 16S rRNA and cytochrome oxidase I (COI) regions. Although no divergence was found between the broodstocks for the highly conservative 16S rRNA gene, 8.2 % distance between *L. vannamei* and the *F. subtilis* was found. Analyses of the COI region showed genetic distances of only 0.2–1 % between the broodstocks, which is not in agreement with the 10.9 % mean distance found between *L. vannamei* and *F. subtilis*. However, it is probable that the small genetic distance values between the five broodstocks studied were not due to mutations that occurred at the time of their foundation but may instead be related to random genetic drift or a founder effects that

occurred during the establishment of the broodstocks. Since mitochondrial genome is maternally inherited without recombination, it is to be expected that variation in this genome is highly susceptible to stochastic events such as genetic drift (Bembo et al. 1995). The broodstocks investigated have been formed by specimens collected from different localities such as Panama and Ecuador, and such a divergence, even if only small, may be reflecting the genetic variation present in the natural populations. However, it is well established that adult *L. vannamei* mate offshore where their planktonic larvae are submitted to the random effects of winds and tides (Benzie 2000), and a large genetically homogeneous and panmictic population is to be expected.

Genetic diversity between wild and cultured penaeids

The genetic variation between wild and cultured populations of *Masupenaus japonicus* is 0.6701–0.8989, and a high polymorphism in all the six microsatellite loci was observed. In this study, more (73) alleles were detected from 93 samples, compared with number of alleles observed in allozyme analysis in wild and cultured populations, which shows that microsatellites exhibit higher level of polymorphism. Therefore, microsatellite is more useful for population genetic structure studies than allozyme analysis. Zhuang et al. (2000) and Sbordoni et al. (1986) showed that three wild populations of kuruma prawn had an effective allele number average of 1.05, 1.4 and 1.3 and reported an average heterozygosity of 0.0015, 0.102 and 0.064, respectively, by enzyme analysis. Zhuang et al. (2001a, b) and Song et al. (1999) showed that two wild kuruma prawn populations from Taiwan Strait and Xiamen water area had average heterozygosities of 0.214 and 0.245, respectively, by RAPD analysis. These values are much lower than those obtained in this study using microsatellite markers. The results obtained in the present paper accorded well with the characteristic that microsatellites may detect much higher polymorphic level and also agreed with the microsatellite analysis of *L. vannamei* and *F. chinensis* (Wolfus et al. 1997; Liu et al. 2004). The mean number of allele locus ranged from 9.83 (cultured) to 11.83 (wild). The number of effective alleles varied from 6.86 (cultured) to 8.58 (wild). The average of observed heterozygosity (HO) of populations 0.6935 (cultured) to 0.7370 (wild), and that of expected heterozygosity (HE) varied from 0.8169 (wild) to 0.8209 (cultured). Differences in the total number of alleles and heterozygosity level between the wild population and cultured population were not significant and were small. Thus, compared with the wild population, the cultured population showed little reduction in genetic variation. F_{ST} value (0.0231) between populations also implied that the difference was very small. Zhuang et al. (2000, 2001a, b) found the same result when comparing a wild population of Taiwan Strait with a cultured population of kuruma prawn from Qingdao, China. However, a cultured population of kuruma prawn was reported to have lost variation when compared with a wild population in Fujian, China (Song et al. 1999). Significant deviations from Hardy–Weinberg equilibrium were found in the two populations. Heterozygote deficiencies were responsible for these deviations because all observed heterozygosities were less than the expected heterozygosity at deviation loci. The total number of alleles (71 and 59) ($p = 0.296$) and paired-samples t test of observed heterozygosity and expected heterozygosity implied that there was no significant difference ($p = 50.572$ and 0.891, respectively) between wild and cultured populations. Some rare allele losses occurred in the cultured population. A total of 14 unique alleles were found in the wild population, but only two unique alleles were observed in the cultured population. One of the domestication effects is the loss of rare alleles. The changes in allelic diversity after bottlenecks may be more striking than changes in heterozygosity (Allendorf and

Ryman 1987). The reductions in unique alleles and the total number of alleles from low frequency (0–0.1) suggest that some rare allele losses might have occurred in the cultured population. Although wild population and cultured population showed high variability, there were significant differences in allele frequencies at four of the six microsatellite loci between populations. Since cultured population was from commercial nauplii, which are usually the progeny of 50–80 wild spawners, and was reared for broodstock, small effective population size and selecting force would be responsible for these reductions and differences between populations. Another cause was the possible full-sib relationship between some animals in the cultured population. While we randomly sampled the cultured specimens from the population, we could not exclude the full-sib possibility unless their parentage and relatedness determination was performed. If this relationship existed, it was a reasonable explanation for the loss of rare alleles. Then, all these indicated that there were some differences between populations, and effective management mechanism would be necessary to avoid losses of genetic variation in the long term. The results obtained in this study revealed that microsatellite marker was a powerful approach to monitor the genetic difference between wild and cultured populations. The cultured population showed little reduction in genetic variation compared with the wild population, but some rare allele losses might have occurred in the cultured population. Therefore, there is a need to improve the hatchery program for preserving the genetic variation of the cultured population.

Next-generation sequencing

Next-generation sequencing (NGS) has revolutionized biologic research and opened up the field of genomics for small-scale projects in non-model organisms (Hudson 2008; Shendure and Ji 2008; Ekblom and Galindo 2011). A practical approach for the studies of species with no prior genomic information available, and where budgets are limited, is to sequence only the expressed parts of the genomes (transcriptomes) known as RNA-Seq (Wang et al. 2009) and has the advantage that sequence characterization is focused on functionally important regions of the genomes. NGS technology has facilitated the collection of large amount of nucleotide information in sequence read length from 30 to 1,500 nucleotides (nt) for hundreds of thousands to millions of DNA molecules simultaneously (Nowrousian 2010). Over the past decade, the cost of NGS has decreased significantly, making it possible to use non-model fish species to investigate emerging environmental issues. Using NGS technology, large amount of raw data can be obtained within short periods of time, thereby enhancing the researches (Mehinto et al. 2012). An additional benefit is that information is obtained not only about gene sequence variation but also regarding gene expression levels ('t Hoen et al. 2008). The most widely used NGS method for de novo characterization of transcriptomes of non-model organisms is the Roche 454 sequencing technology. Data from small-scale RNA-Seq studies are routinely used for a number of different purposes such as gene finding, marker identification and expression studies (Ekblom and Galindo 2011). RNA-Seq studies can also address questions about differential expression between different tissues, life stages, individuals or populations, differences that may be important for understanding gene function, development, phenotypic plasticity, local adaptation and speciation (Barakat et al. 2009; Kristiansson et al. 2009; Wolf et al. 2010). A fraction of a sequencing run may enable genetic variation to be achieved efficiently in non-model organisms (Ekblom and Galindo 2011). NGS transcriptome data have been extensively utilized for identifying microsatellites, also known as SSRs , in non-model organisms (Cheung et al. 2008; Hahn et al. 2009; Der et al. 2011).

Currently, Illumina sequencing produces short reads of about 100 nt in length but has the ability to do this from each end of the DNA molecule when paired ends are used. The SOLiD likewise produces reads of approximately 35–60 nt in length. The short sequences yielded by Illumina and SOLiD platforms have proved to be useful for the detection of miRNA (small RNA molecules of about 22 nt) (corroborates with Chi et al. 2011; Johansen et al. 2011) and comparative genome analysis of different fish populations (Chi et al. 2011). Development of NGS technologies allows the acquisition of more sequence data per run at a substantially lower cost than in long-read technologies (Mardis 2008). Yet, because of the short read lengths, application of NGS technologies has generally been restricted to model organisms for which the genome sequences are already known. However, recent algorithmic and experimental advances have made it possible to succeed at de novo sequence projects (Li et al. 2010; Dalloul et al. 2010). Complexity in transcriptome analyses is reduced rather than those in genomic one, which are receiving attention as they are likely to be suitable for discovering some expressed genes, SNPs and microsatellite regions in non-model organisms (Kawahara-Miki et al. 2011). Gibbons et al. (2009) suggested the utilities of short-read sequencing for evolutionary studies on tropical disease vectors *Aedes aegypti* and *Anopheles gambiae*. Furthermore, species differentiation of crow species *Corvus corone* and *Corvus cornix* was detected through differences in their gene expression profiles, whereas the use of several DNA markers failed to detect this differentiation (Wolf et al. 2010). In mammals, transcriptome analyses in the Antarctic fur seal *Arctocephalus gazella* using mRNA derived from skin tissues detected several thousand putative microsatellite loci and SNPs (Hoffman 2011). These pioneering studies have authenticated that NGS provides a massive amount of valid information for population genetics in non-model organisms. Recently, de novo transcriptome analyses, that is, de novo assembly of short reads from mRNA without genome reference, have emerged. Several studies have reported the transcriptome sequencing of various non-model species using NGS technologies. Kawahara-Miki et al. (2011) tested the utility of NGS technologies for studying the egg phenotypic variations between two populations of a non-model species such as the Hokkai shrimp (*Pandalus latirostris*; Decapoda, Pandalidae), NTK and NTR (*P. latirostris* collected at the lagoons of Notsuke Bay and Lake Notoro in Hokkaido, Japan). They showed the results of transcriptome analysis using mRNA extracted from the ovaries of two females in each population gene differentially expressed between them. The de novo assembly approach for the transcriptome of a non-model species using only short-read sequence data showed a strategy for identifying sequences with different expression levels between the two populations of Hokkai shrimps. Therefore, we conclude that NGS technologies will provide new insights into the evolutionary study of phenotypic variation in wild organisms. Whole-genome sequencing is the most robust method to identify the great variety of genetic diversity in a population and gain a greater understanding of the relationship between the inherited genome and observed heritable trait. Genome sequencing technology is the standard method for genetic polymorphism discovery in non-model organisms (Duran et al. 2009).

Conclusions

The dramatic development of molecular genetics since the first widespread use of allozymes in the 1970s, currently exemplified by the Human Genome Project and other equally ambitious undertakings, has laid the groundwork for genomics. The development of DNA-based genetic markers had a revolutionary impact on animal genetics. Knowledge on

molecular genetics has been applied to the genetic characterization of the populations, covering a variety of aspects, with special emphasis on diversity analysis and conservation. With the increasing global demand for aquaculture products and the early stage of selection for most aquatic species, molecular genetics is expected to play a pivotal role in the management of breeding programs aimed at developing improved strains for the most economically important species. Genetic tagging and population studies such as RFLP and RAPD markers are used to differentiate between wild and cultured shrimp populations and families useful in breeding. Molecular markers can be used for the development of genetic maps, including RFLP, RAPD, AFLP and SSR. Considerable progress can be expected from gene mapping in the future. The efforts are devoted to the enrichment of the genetic maps and integration of genetic linkage and physical maps, which is essential for the understanding of genes responsible for performance traits, including growth and disease resistance. Moreover, every technology has its own limitations; hence, further research should be made to rectify the stumbling blocks that hinder accessing the genetic variation with accuracy.

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