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Research Article

PCR-based VNTR core sequence analysis for inferring genetic diversity in the shrimp *Litopenaeus vannamei*

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Abstract

The genetic variation in two farmed strains (F_3-Panama and F_{17}-Venezuela) of the shrimp *Litopenaeus vannamei* was examined based on DNA multiloci analyses. Eighteen adults of each strain were analyzed by PCR using a set of VNTR core sequence primers. Genetic similarity, mean allele frequency, mean heterozygosity and the frequency of polymorphic loci were determined for both strains. A dendrogram of genetic similarity was produced by UPGMA clustering. The results for three primers (INS, M13, YN73) revealed different levels of genetic variation within the strains. The higher genetic similarity seen within strain F_{17} was apparently related to inbreeding, although a bottleneck effect could not be discarded. The low level of genetic variability of this strain could account for the reduced adaptive advantage of these animals and their inability to adjust to breeding conditions in Brazil.

Key words: Litopenaeus vannamei, genetic diversity, shrimp, VNTR, PCR.

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Introduction

Marine shrimp farming in Brazil has increased since the introduction of the species *Litopenaeus vannamei* from the Pacific Ocean. Currently, there are about 500 shrimp farms throughout Brazil, particularly in the northeastern region, with nearly 100% of all reared shrimp being *L. vannamei* (ABCC, 2002).

Despite several advantages, rearing exotic species can have undesirable consequences in a production system. The higher incidence of dwarf shrimps seen during harvesting may reflect genetic erosion within cultured stocks (Benzie *et al*., 1992, 1993). Bottleneck effects, often produced by the reduced number of broodstock used in each generation, can lead to decreased genetic variability and to the loss of rare alleles at certain loci (Perez and Romero, 1991). In addition, inbreeding progressively reduces the genetic variability and this can lead to a decrease in the mean size of the shrimps or to incomplete embryo development (Sunden and Davis, 1991). A close relationship between reduced genetic variability and a decline in spawning capacity has been reported for *Marsupenaeus japonicus* (Sbordoni *et al.,* 1986).

In the past, enzymatic analyses were essential for initiating genetic studies on commercially-reared shrimps (Villaescusa *et al*., 1984; Alonso *et al*., 1987; Espinosa *et al*., 1989; Labacena *et al*., 1994; Díaz *et al*., 1995).

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However, more recently, various techniques in molecular biology have been used to characterize the genetic diversity and to establish DNA markers in several penaeid species (Garcia *et al*., 1996; Wolfus *et al*., 1997; Tassanakajon *et al*., 1997; Moore *et al*., 1999).

Restriction fragment length polymorphism (RFLP) and random amplified polymorphism DNA (RAPD) analyses have been used to assess the genetic diversity among different cultured stocks of *L. vannamei* shrimps (Garcia *et al*., 1996). In addition, microsatellite analyses have revealed specific genetic markers in *L. vannamei* and were able to detect different levels of genetic variation among wild populations (Wolfus *et al.*, 1997). Genetic differences among cultured prawns *L. vannamei* (Garcia *et al.*, 1996) and *Penaeus monodon* (Tassanakajon *et al.*, 1997) have been reported based on the multiloci patterns obtained in RAPD analyses. Similarly, multiloci PCR-based VNTR (variable number of tandem repeats) analyses can be useful for assessing genetic diversity within and between wild species (Heath *et al*., 1993) and cultured strains (Bagshaw and Buckholt, 1995).

In the present work, PCR-based analyses using a set of VNTR core sequence primers were done to assess the genetic variability within and between two strains of *L. vannamei,* which have been farmed in Brazil for different numbers of generations.

Material and Methods

Samples were obtained from 18 F_3 individuals of a strain owned by EMPARN (Empresa de Pesquisa

Agropecuária do Rio Grande do Norte, Natal, RN), the F_0 parents of which originally came from the Panama coast, and from 18 F_{17} individuals imported by AQUATEC Hatchery (Canguaretama, RN) from a Venezuelan shrimp farm. Genomic DNA was extracted from the hepatopancreas (frozen in liquid nitrogen) using phenol:chloroform: isoamyl alcohol (25:24:1, v/v) as described by Sambrook *et al*. (1989).

PCR was done using five universal VNTR core sequence primers (INS: 5' ACAGGGGTGTGGG 3', YNZ22: 5' CTCTGGGTGTCGTGC 3', YN73: 5' CCCG TGGGGCCGCCG 3', M13: 5' GAGGGTGGNGGNTCT 3' and PER1: 5' GACNGGNACNGG 3') selected from Heath *et al*. (1993). Each DNA amplification reaction used 200 ng of prawn genomic DNA in a total reaction volume of 50 µL containing 1x Cetus buffer (Gibco), 1.5 mM $MgCl_2$, 0,2 mM of each dNTP, 500 ng of primer and 1 unit of *Taq* DNA polymerase (Gibco). The reactions were done in a Perkin Elmer Cetus DNA thermal cycler (model 2400) and involved denaturation for 3 min at 95 °C followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, and a final extension at 72 °C for 3 min. The amplification products were analyzed by electrophoresis in 12% polyacrylamide gels (5 h at 80 V) and detected by silver nitrate staining (Sambrook *et al*., 1989). The fragment patterns of individuals were scored based on the absence or presence of bands, assuming that alleles from different loci did not co-migrate to the same position and that each fragment represented a Mendelian locus in which the visible dominant allele was in Hardy-Weinberg equilibrium with a recessive null allele or absent fragment (Lynch and Milligan, 1994).

The similarity coefficient (S_j) (Jaccard, 1901) and a dendrogram of genetic similarity produced by UPGMA (the unweighted pair-group method with arithmetic averages) clustering method were obtained using the NTSYS-PC program (Rohlf, 1988). The mean allele frequency ($q = 1 - \sqrt{(1-Sj)}$) (Jeffreys and Morton, 1987) and the mean heterozygosity ($h_e = 2(1-q)/(2-q)$) (Georges *et al*., 1988), both of which have been used successfully in RAPD analyses (Wasko and Galetti Junior, 2002) in which heterozygotic individuals could not be identified, and the frequency of polymorphic loci ($f = L_p/L$) (Ford, 1940), where L_p = number of polymorphic loci and L = total number of loci, calculated for each primer, were determined.

Results

Electrophoretic analyses using the primers YN73, INS and M13 provided reliable, distinct multiloci band profiles for the two strains (Figure 1); no PCR products were obtained with PER1 and YNZ22. Twenty-seven loci 200-1000 bp long were analyzed. Jaccard's genetic similarity index showed higher values among the F_{17} individuals of the Venezuela lineage than for F_3 of the Panama strain, even when the results for each primer were considered separately (Table I). All F_{17} individuals from the Venezuela strain were clustered at nearly 90% of genetic similarity, whereas the F_3 individuals from the Panama strain had lower similarity values (Figure 2). Accordingly, the observed mean heterozygosity and the polymorphic loci frequencies were higher for F_3 of the Panama strain, whereas the mean allele frequency was higher in F_{17} individuals of the Venezuela strain (Table II).

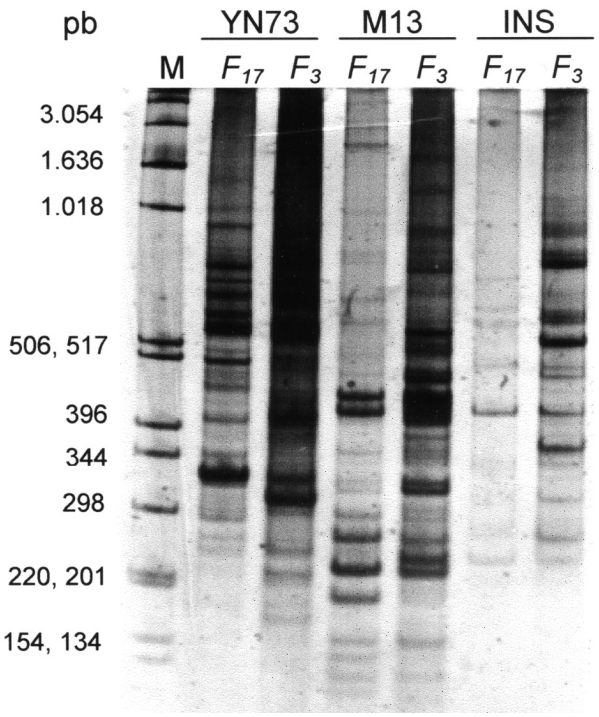

Figure 1 - Multiloci profiles obtained with the VNTR primers M13, YN73 and INS in two strains (F_3 Panama and F_{17} Venezuela) of *L. vannamei*.

Discussion

L. vannamei is one of the main marine shrimp species used in commercial farming worldwide, especially in the American continent (Guerrelhas, 1997). There are extensive coastal areas dedicated to farming this species in Pan-

Table I - Jaccard's genetic similarity index (Sj) for both strains of *L. vannamei* (F_3 Panama and F_{17} Venezuela). The similarity index was obtained by considering each VNTR primer (M13, YN73, INS) separately, and then pooling the data.

Jaccards Genetic Similarity	Strains	
	F_3	F_{17}
$S_{j\,(M13)}$	0.679	0.888
$S_{j\,(YN73)}$	0.697	0.944
$S_{j\,(INS)}$	0.372	0.951
$Sj_{\,(M13, YN73, INS)}$	0.56	0.91

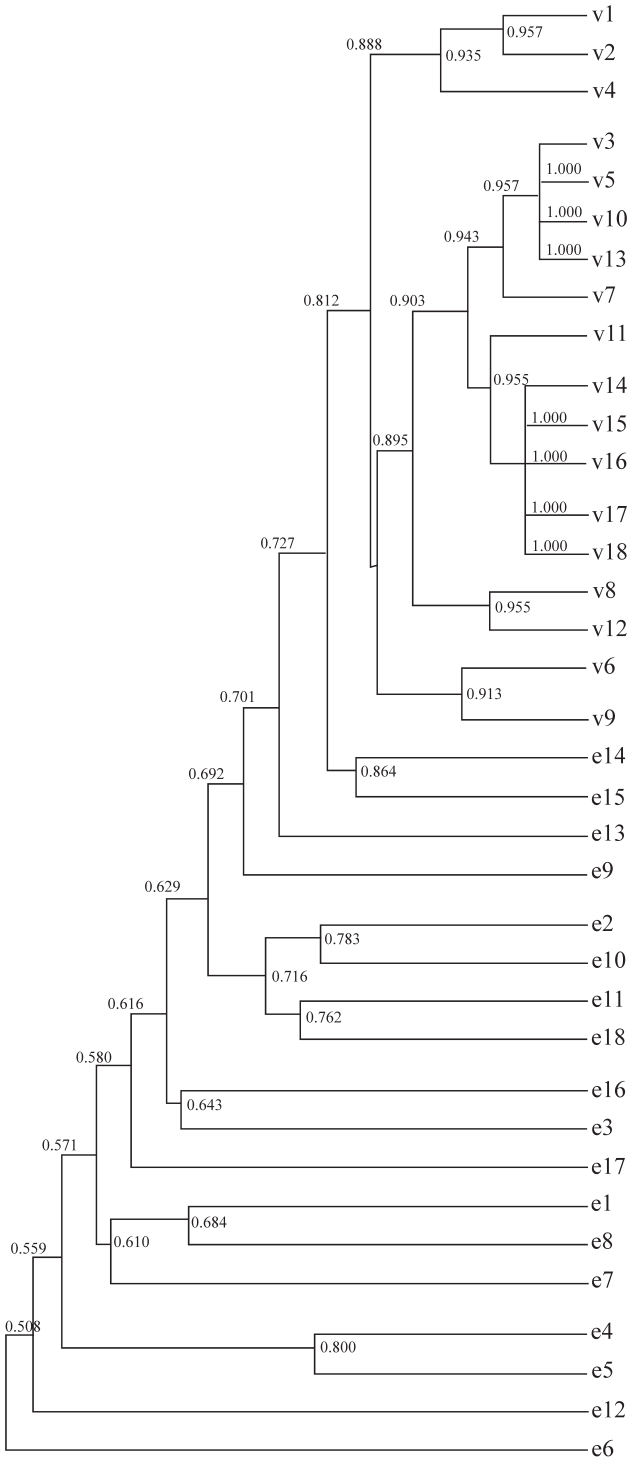

Figure 2 - Dendrogram of genetic similarity for individuals of F_3 Panama (e_1 - e_{18}) and F_{17} Venezuela (v_1 - v_{18}) based on the UPGMA method.

ama and Ecuador. Wild populations of *L. vannamei* do not occur on the Atlantic coast and farming of this species in Brazil was initially supported on the importation of specimens (larvae or adult stock). Currently, various farm-reared broodstock of this species are available from several Brazilian larvae-producing centers.

Table II - Frequency of polymorphic loci (*f*), allele frequency (*q*) and mean heterozygosity (h_e) in F_3 Panama and F_{17} Venezuela strains based on the VNTR primers M13, YN73, INS.

Values	Strains	
	F_3	F_{17}
$f_{(M13)}$	0.89	0.44
$f_{(YN73)}$	0.67	0.11
$f_{(INS)}$	1.00	0.11
q	0.34	0.70
h_e	0.795	0.461

Species domestication can provide individuals more adapted to captive conditions, but also can lead to genetic losses (Wolfus *et al.,* 1997). In agreement with this, the multiloci patterns obtained in the PCR-based VNTR analyses suggested that the F_{17} Venezuela strain consisted essentially of genetically very similar individuals when compared with Panama F_3 individuals. The higher similarity scores among F_{17} individuals appeared to reflect to their higher level of consanguinity, which probably resulted from inbreeding over generations. Genetic drift produced by mating a reduced number of individuals in each generation may also be present. As shown elsewhere, a bottleneck effect due to genetic drift could be the most important cause of the loss of genetic variation in inbred strains of *M. japonicus* (Sbordoni *et al*., 1986).

In contrast, individuals of the Panama (F_3) strain retained a wider genetic potential. The dendrogram of genetic similarity scores showed that individuals of this strain were genetically more distant from each other than were the individuals from the F_{17} Venezuela strain. The higher genetic variation in the Panama strain could provide these shrimps with an advantage in adapting to different local environmental conditions.

The Venezuela F_{17} individuals were originally imported by a Brazilian breeder because of their high resistance to disease, good growth performance and reproductive profile. It was thought that these animals would do as well in Brazil as in their country of origin. However, this strain was adapted poorly to Brazilian conditions, and was highly susceptible to widespread viral diseases (Guerrelhas, personal communication). In agreement with this finding, SPF (specific pathogen free) strains of *L. vannamei* may vary appreciably in their susceptibility to disease and in their growth performance (Carr *et al*., 1994), thus limiting their use worldwide. Molecular analyses using microsatellite markers detected decreased mean heterozygosity (h_e) in SPF shrimps (Wolfus *et al*., 1997). In the latter study, domesticated populations from Ecuador showed a marked reduction in genetic diversity in the F_7 generation (h_e = 0.45) when compared to wild populations (h_e = 1.00). These authors believed that without adequate monitoring of the levels of genetic variation, the genetic diversity in F_7 individuals could be com-

pletely lost in the subsequent generations. The low levels of genetic variability seen in F_{17} individuals of the Venezuela strain could be responsible for the reduced adaptive advantage of these animals and their inability to adjust to breeding conditions in Brazil.

According to the Brazilian Association of Shrimp Farmers (Associação Brasileira de Criadores de Camarão), there are about 22 hatcheries in the country, mainly in the states of Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Bahia and Santa Catarina, and different inbred broodstock are currently used to produce either nauplii or post-larval individuals (ABCC, 2002). Assessing the genetic variability in such strains could help in deciding the appropriate time to introduce a new genetic pool (by mating genetically distant strains) and could contribute significantly to the development of genetic improvement programs. A knowledge of the genetic structure of the shrimp broodstock strain is an important step towards more predictable and sustainable shrimp farming.

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