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Short Communication

Fluorescent amplified fragment length polymorphism (fAFLP) analyses and genetic diversity in *Litopenaeus vannamei* (Penaeidae)

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Abstract

The Pacific white shrimp, *Litopenaeus vannamei* (Penaeidae), represents about 95% of all Brazilian shrimp production. The Brazilian *L. vannamei* foundation broodstock was made up of specimens collected from different American Pacific sites, but little information was collected on the genetic structure of the broodstock. We used the fluorescence amplified fragment length polymorphism (fAFLP) method to study the genetic diversity of *L. vannamei* broodstock lines 03CMF1 and 03CBF1 originally produced by breeder-shrimps imported mainly from Panama and Ecuador, although wild individuals from other localities may also have been used in producing these two lines. Our results showed a total of 93 polymorphic bands ranging from 50 to 500 bp, the mean Nei's genetic diversity calculated for the total sample was 13.4% and identity and genetic distance analyses indicated high genetic homogeneity within and between both the broodstock lineages studied which suggests that they had similar genetic structure. These results may represent an important tool for the appropriate management of *L. vannamei* broodstocks.

Key words: fAFLP, genetic diversity, *Litopenaeus vannamei,* Penaeidae, shrimp.

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The Pacific white shrimp, *Litopenaeus vannamei* (Penaeidae), represents about 95% of all Brazilian shrimp production which has risen from 40,000 ton in 2001 to approximately 80,000 ton in 2003, representing nearly 5.3% of world production and making Brazil among the top seven producers (Rocha and Rodrigues, 2003).

The *L. vannamei* foundation broodstock was created from specimens collected from different American Pacific sites and little is known on their genetic structure. Genetic tools consisting of molecular and genetic manipulation technologies have been widely used to improve the aquaculture of several species (Dall *et al*., 1990, Sagi and Cohen, 1990, Xiang *et al.*, 1992), with polymerase chain reaction (PCR) technology having been particularly useful in identifying several molecular markers which have been extremely useful in shrimp aquaculture where they have been used for the identification of populations and genetic diversity analyses (Sunden and Davis, 1991; Garcia *et al*., 1996).

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The fluorescence amplified fragment length polymorphism (fAFLP) assay is based on the amplification of restriction fragments from genomic DNA and the analysis of the amplified products using polyacrylamide gel electrophoresis, differing from conventional AFLP only by the use of primers labeled with a fluorochrome instead of the radioactive phosphorus [γ^{32} P] used in the standard method. Conventional AFLP is particularly useful for genomic mapping and genetic fingerprinting and may be used to calculate genetic distances between individual members of a population (Vos *et al.,* 1995), AFLP markers having already been used for constructing linkage maps, establishing pedigrees and identifying quantitative trait loci (QTL) in shrimp species such as *Marsupenaeus japonicus* (Moore *et al.,* 1999; Li *et al.*, 2003) and *Penaeus monodon* (Wilson *et al*., 2002). Garcia *et al.* (1994) used allozymes, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) analyses to study the genetic diversity of cultured *L. vannamei* and detected greater genetic variation using RFLP as compared to RAPD, although RAPD has been used to assess genetic variation in six captive shrimp families (Garcia and Benzie, 1995) and in wild

populations of *P. monodon* (Tassanakajon *et al.,* 1997), and in *Litopenaeus stylirostris* (Aubert and Lightner, 2000).

Genetic studies in captive Brazilian *L. vannamei* populations have only recently been carried out, Freitas and Galetti Jr. (2002) having used PCR-based variable number tandem repeat (VNTR) core sequence analysis to detect significant genetic differences between two reared lineages, while Freitas (2003) used RAPD analysis to study the genetic diversity of 15 broodstock lines owned by seven Brazilian hatcheries and Francisco (2003) applied mitochondrial DNA analyses to reveal low genetic distances among four reared broodstocks.

The study presented in this paper was carried out in order to standardize a fAFLP protocol for studying shrimp DNA, and to evaluate the genetic diversity within and between two *L. vannamei* broodstocks reared in northeastern Brazil.

Samples were obtained from broodstock lines 03CMF1 (16 specimens) and 03CBF1 (14 specimens) (both lines owned by Compescal hatchery industry, Aracati, Ceará, Brazil) which had originally been formed from by breeder-shrimps (about 100 breeder couples for each line) imported mainly from Panama and Ecuador, although wild specimens from other localities may also have been incorporated into the stocks.

Total genomic DNA samples were obtained from the pleopod muscles of each specimen using a phenol/chloroform/isoamyl 25:24:1 protocol (Sambrook *et al.,* 1989). The fAFLP reactions were performed using to the Applied Biosystems AFLP Plant Mapping Protocol Kit (Anon, 1997) with the genomic DNA (500 ng) being digested with *Eco*RI and *Mse*I before ligation of restriction site-specific adaptors. Pre-selective amplification was carried out using adaptor-specific primers with a single selective base on each primer, two sets of site-specific *Mse*I and *Eco* RI primers (ACG-CAA and AGG-CAG) being used for the selective amplification reaction. Each sample was loaded onto a 5% Long-Ranger denaturing gel which was run in an ABI PRISM^{TM} 377 DNA Sequencer (PE-Applied Biosystems) for 3 h at 2,500 V. The electrophoresis images obtained from each run were analyzed using the GENESCAN program version 3.1 (Applied Biosystems). The Genotyper program version 2.5 (Applied Biosystems) was used to verify the presence (coded as 1) or absence (coded as 0) of polymorphic bands in the electropherograms and produce a binary matrix and the POPGENE program version 1.31 (Yeh *et al*., 1999) was used to calculate Nei's genetic diversity (Nei, 1973), genetic distance (Nei, 1972) and identity (Nei, 1978).

We found that the fAFLP method was efficient at analyzing genetic diversity in *L. vannamei* broodstocks. The banding-patterns obtained with the two primer pairs for the 30 DNA samples generated a total of 93 polymorphic bands with sizes ranging from 50 to 500 bp. The fluorescent ACG-CAA primer pair generated 48 polymorphic bands, while the AGG-CAG primer pair generated 45 polymorphic bands.

Nei's genetic identity value between both broodstock lines was 0.9947 and Nei's genetic distance was 0.0053, suggesting that the genetic structure of the two lines are very similar. The historical data for the two lines show that the majority of the founder-shrimps came from Panama and Ecuador, the genetic homogeneity observed by us possibly being due to this common origin. Both broodstocks were F_1 lines (separated from each other by only one generation), so if genetic drift occurred during the foundation of the F_1 lines it may have had quite similar effects on the genetic structure of both lines. Mitochondrial DNA (mtDNA) analysis has also shown low genetic divergence and an inferred common origin for other *L. vannamei* broodstock lines (Francisco, 2003).

It is well-known that molecular analyses are very important in order to conduct an adequate monitoring of captive stocks and to establish profitable genetic improvement programs (Sunden and Davis, 1991). Reduction in genetic diversity may increase susceptibility to disease and other selective factors which can lead to lower populations (Xu *et al.,* 2001). Genetic deterioration of cultured shrimp populations has been related to inbreeding effects in succeeding generations and genetic drift. Sbordoni *et al*. (1986) analyzed the genetic variability of *Penaeus japonicus* cultured for six generations and found a continuous reduction in the level of genetic variability with each succeeding generation which they thought was a consequence of severe genetic drift and inbreeding. Freitas (2003) also reported such effects in *L. vannamei* where RAPD analyses revealed significant loss in genetic variation during five inbred generations. It has been stated (Waldman, 1999) that the increase in intrapopulational genetic similarity during succeeding generations results in a large interpopulational genetic distance, and it is also known that genetic variation can be restored by using separated inbred lines for cross-breeding (Freitas and Galetti Jr., 2002; Sánchez *et al*., 2003).

When both broodstock lines were grouped together we found that the overall mean Nei's genetic diversity (13.4%) was still not high (Table 1) as compared to the levels detected in an AFLP study of wild *L. vannamei* populations by Travis (2002) who found higher levels of genetic variation than those found in our studies. Although effects related to the relatively small size of our sample (100 couples) should be borne in mind, we believe that the apparent reduction in genetic variation observed by us was probably due to genetic drift during broodstock foundation.

Our results contribute data on the study of genetic variation in captive populations of shrimps using fAFLP and show that both the broodstock lines studied have a very similar genetic structure, the assessment of genetic structure representing an important tool for designing more appropriate management programs for *L. vannamei* broodstocks.

Table 1 - Nei's genetic diversity values for the total *Litopenaeus vannamei* sample (n = 16 for broodstock line 03CMF1; n = 14 for line 03CBF1) and the overall sample mean (n = 30) and the overall mean considering both sets of primers.

Fragments from ACG-CAA primer	Nei's genetic diversity	Fragments from AGG-CAG primer	Nei's genetic diversity
1a	0.1286	1b	0.0333
2a	0.0655	2b	0.1286
3a	0.3032	3b	0.0976
4a	0.1295	4b	0.2527
5a	0.3296	5b	0.3296
6a	0.3020	6b	0.1596
7a	0.0655	7b	0.4142
8a	0.1295	8b	0.0978
9a	0.1327	9b	0.3020
10a	0.2185	10b	0.2741
11a	0.1889	11b	0.2775
12a	0.0333	12b	0.2998
13a	0.1625	13b	0.0978
14a	0.0655	14b	0.3002
15a	0.2481	15b	0.2998
16a	0.2467	16b	0.1286
17a	0.1889	17b	0.3494
18a	0.2775	18b	0.2775
19a	0.1286	19b	0.2481
20a	0.1295	20b	0.0655
21a	0.1889	21b	0.0655
22a	0.0655	22b	0.0668
23a	0.0333	23b	0.2775
24a	0.2734	24b	0.0978
25a	0.0333	25b	0.1341
26a	0.0333	26b	0.0655
27a	0.0333	27b	0.2217
28a	0.0334	28b	0.0976
29a	0.0334	29b	0.0655
30a	0.0333	30b	0.0333
31a	0.2461	31b	0.0655
32a	0.0334	32b	0.0333
33a	0.0333	33b	0.1301
34a	0.0655	34b	0.0333
35a	0.0333	35b	0.0333
36a	0.0333	36b	0.1889
37a	0.0334	37b	0.0333
38a	0.0655	38b	0.0333
39a	0.0333	39b	0.2185
40a	0.0334	40b	0.0333
41a	0.0333	41b	0.0333
42a	0.2775	42b	0.0334
43a	0.1906	43b	0.0333
44a	0.0655	44b	0.0334
45a	0.2481	45b	0.0668
46a	0.0978		
47a	0.0976		
48a	0.0333		
Overall mean			0.1339

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