Microsatellites from the White Shrimp Litopenaeus schmitti (Crustacea, Decapoda)

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Introduction

Litopenaeus schmitti is one of the two economically relevant shrimp species in Cuba; particularly for its introduction in aquaculture more than fifteen years ago. Different human activities and natural events have deeply affected shrimp ecosystems endangering natural populations of this species. One way to protect this resource and indirectly sustain shrimp aquaculture industry is to evaluate genetic variability and natural population structure [1]. Previous works using allozymes have suggested a limited genetic variability among penaeids populations at limited geographic scales [2, 3]. However, a higher sensitivity and a discriminatory power have been described using other molecular markers such as mtDNA, RAPDs, and microsatellites, when studying the population genetics of different penaeid species [4–7]. Especially, a growing body of information using microsatellite sequences is available for some penaeids, and a number of primer sets for PCR amplifications has been designed [6, 8–10]. However, the usefulness of the primers for inter-species application has been questioned in some studies [11]. The aim of the present study is to characterize microsatellites loci isolated from L. schmitti and evaluate the usefulness of two of them in genetic population studies.

Materials and Methods

Library construction and screening

Genomic DNA was extracted from a single ethanol preserved pleopod muscle of L. schmitti according to García-Machado et al. [12]. Partial genome library construction and screening was carried out following Estoup and Cornuet [13]. Sac3AI restriction fragments of sizes ranging from 300 pb to 700 pb were ligated to a previously BamHI-digested and alkaline phoshatase-treated pUC18 vector. Ligations were used to transform Epicurium Coli® XL1-Blue ultracompetent cells (Stratagene, USA).

All the colonies were screened for repetitions using the DIG-labeled oligonucleotides (TC)₅, (TG)₅, (TAA)₁₅ and (GTG)₁₅. Labeling was achieved using the Tailing Kit (Boehringer Mannheim, Germany). The (TC)₅, (TG)₅ and (TAA)₁₅ oligonucleotides were pooled in the same hybridization mix, and the hybridization temperature was set to 48 °C, whereas hybrid-
ization temperature for (GTG), oligonucleotide was 65 °C. Complete or partial sequences from 30 positive clones were obtained using an automatic sequencer ALF express (Amersham Pharmacia-Biotech, Sweden), and using as sequencing primers the universals M13-40 forward and M13 reverse primers.

DNA was isolated as described above from samples of three populations. Different amplification conditions were assayed with unique pair of primers designed for each locus, changing MgCl₂, DNA, and primer concentrations and the annealing temperature (Table). PCR products were run on 6% sequencing gels and scored against a known sequence. The corresponding bands were developed using the Silver Stain Sequencing Kit (Promega, USA).

The deviations from Hardy–Weinberg equilibrium were estimated using the program Byosis [14]. The observed and expected heterozygosity, the number of alleles per locus and the linkage disequilibrium and the observed and expected heterozigosity, the number of alleles was different at each population. Yaguanabo, one of the hatchery populations, presented only 2 Lsch-1 alleles and 5 Lsch-2 alleles (Figure). This result could be expected as genetic variation is not taken into account during stock foundation and maintenance across

Allele frequencies for the two loci are shown in the Figure. The size of the Lsch-1 alleles seems to vary according to the repetition of two bases. Two variants, differing by four nucleotides, were far more frequent in locus Lsch-2. However, other alleles appear apparently resulting from the loss or gain of 1, 2 or 3 bases. They could be related to the presence of 4 or 5 T instead of 3 in some places, since such sequences are observed once each in the sequenced clone. For this reason the variation of the Lsch-2 locus was not well defined as a change of the repetition number.

The Table summarizes the main characteristics and some variability estimates of the two-microsatellite markers from the studied populations. The quantity of alleles was different at each population. Yaguanabo, one of the hatchery populations, presented only 2 Lsch-1 alleles and 5 Lsch-2 alleles (Figure). This result could be expected as genetic variation is not taken into account during stock foundation and maintenance across

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif</th>
<th>Size range (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Relative diversity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lsch-1</td>
<td>(GT)₁₀</td>
<td>136-206</td>
<td>60</td>
<td>0.59 / 0.57</td>
</tr>
<tr>
<td>Lsch-2</td>
<td>(CTT)₁₀ (CTT)</td>
<td>208-215</td>
<td>50</td>
<td>0.43 / 0.58</td>
</tr>
</tbody>
</table>

Table. Primer, repeat motif, amplification conditions and results of amplification of two microsatellite loci in three populations of L. schmitti.

Figure. Allele sizes (bp) and frequencies at each Litopenaeus schmitti populations. A) Lsch-1; B) Lsch-2.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif</th>
<th>Type of repeat</th>
<th>Size range (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Relative diversity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lsch-1</td>
<td>(GT)₁₀</td>
<td>Perfect</td>
<td>136-206</td>
<td>60</td>
<td>0.59 / 0.57</td>
</tr>
<tr>
<td>Lsch-2</td>
<td>(CTT)₁₀ (CTT)</td>
<td>Imperfect-composite</td>
<td>208-215</td>
<td>50</td>
<td>0.43 / 0.58</td>
</tr>
</tbody>
</table>

Primer sequences were: Lsch-1, 5'-GGGCTCATTAGGGCTTTCCTCTCAG-3' and 5'-GAGGTGGTACATATTGGACGCACGC-3'; Lsch-2, 5'-TAAAGCTGCAATTGTCACGTG-3' and 5'-TTAGCTATTGAACCGAATG-3'.

Samples from wild population; *samples from hatchery populations.
Microsatellites from *Litopenaeus schmitti*
generations, which result in most cases in a bottleneck effect and inbreeding [21].

Santa Cruz population is the first generation from wild brood stock reared at the hatchery center. The allele number of this population is similar to Tunas de Zaza’s wild population. This type of domesticate group of animals has been reported with a higher heterozigosity of some isozyme loci and a particular behavior of number of rostral tooth [22].

Relative diversity was higher for locus *Lsch-1* (Table) indicating stronger resolution of populations. This can be related with a higher variation of dinucleotide repeat; according with the results of Chakraborty *et al.* [23] that found a high mutation rate in a survey of microsatellite variability in natural populations.

The difference between the observed and expected heterozigosity was higher for *Lsch-2* than *Lsch-1*, but no significant departure from the Hardy-Weinberg equilibrium for both loci in all three populations and no linkage disequilibrium were found.

Taking into account that both loci resulted polymorphic and that the genetic variability correlates with population histories, it could be suggested that *Lsch-1* and *Lsch-2* are useful markers for the study of *L. schmitti* populations.

Other potentially useful microsatellite loci from the present library are under analysis for making a better characterization of *L. schmitti* wild and cultured populations.

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