Characterization of two microsatellite PCR multiplexes for high throughput genotyping of the Caribbean spiny lobster, *Panulirus argus*

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Abstract

The spiny lobster *Panulirus argus* supports one of the most economically important commercial fisheries in the Caribbean, yet its sustainable management is problematic due to uncertainty regarding levels of population connectivity among Caribbean nations. We developed two microsatellite multiplex panels for *P. argus* to assist in future conservation genetics research studies of this important Caribbean species. Significant deviations from Hardy–Weinberg equilibrium were observed at locus Par7 in multiplex 1 and loci Fwc08 and Fwc17 in multiplex 2. No evidence of linkage disequilibrium was observed. All 12 loci were used in both microsatellite multiplexes were polymorphic, with an average of 12 alleles per locus (ranging from 3 to 29 alleles per locus) and H_O ranging from 0.368 to 0.921. These two microsatellite multiplexes will be a valuable resource for ongoing and future studies of conservation genetics in the Caribbean spiny lobster, *Panulirus argus*.

1. Introduction

The spiny lobster *Panulirus argus* supports one of the most economically important commercial fisheries in the Caribbean, yet its sustainable management is problematic because of its widespread larval dispersal and, consequently, unknown patterns in population connectivity among Caribbean nations (Kough et al. 2013). Polymorphic microsatellite loci with high information content are of great utility for population genetics and connectivity studies. Microsatellite loci have previously been characterized for *P. argus* (Diniz et al. 2004; Tringali et al. 2008), but studies of *P. argus* genetics would benefit from a microsatellite multiplex methodology because it decreases the cost and time required for genotyping individuals while increasing throughput. Our objective was to develop novel microsatellite multiplex panels for *P. argus* to assist in future conservation genetics research studies of this important Caribbean species.

2. Methods

Total genomic DNA was isolated from leg muscle tissue from 56 individuals collected from Caye Caulker, Belize using the Wizard SV-96 Genomic DNA extraction kit following the manufacturer's protocol (Promega). The collection of lobster muscle samples was approved by the Belize Fisheries Department, Approval Number: 00009-09. The polymerase chain reactions (PCRs) were performed in a separated run for each multiplex (Table 1). Each PCR reaction was performed in a total volume of 5 µl using a Veriti thermal cycler (Applied Biosystems). Our methods followed the manufacturer's recommendations (Qiagen Microsatellite

Multiplex PCR Kit), however, the total volume of each PCR reaction was scaled down from 25 µl to 5 µl whilst keeping the concentrations of all PCR reagents the same. The final PCR reaction mix consisted of 0.5 µl of the 10X primer mix (1µM primer + 1µM fluorescent primer), 2.5 µl of Type-it Multiplex PCR Master Mix (Qiagen), 1 µl of molecular grade water and 1µl of (10-20 ng/µl) genomic DNA. The PCR parameters consisted of an initial denaturation at 95 °C for 5 min, followed by 26 cycles at 95 °C for 30 s, 57 °C for 120 s, and 72 °C for 30 s. This was followed by final extension at 60 °C for 30 min. The PCR products were detected on an ABI 3730xl Sequencer (Applied Biosystems) at the University of Manchester DNA sequencing facility. The resulting microsatellite fragments were examined using GENEMAPPER 3.7 (Applied Biosystems) and peaks were scored manually. Any primer pairs that failed to amplify or were difficult to score due to excessive stuttering or split peaks were discarded and not used in further analyses. Microsatellite alleles were binned and error checking was preformed using the R package MsatAllele (Alberto 2009). The R-package POPGENREPORTS was used to estimate observed (H_O) and expected (H_E) heterozygosity, number of alleles (N_A), and deviations from Hardy–Weinberg equilibrium. Bonferroni corrections were applied in POPGENREPORTS when multiple statistical tests were conducted. The program MICROCHECKER (van Oosterhout et al. 2004) was used to check for null alleles and scoring errors caused by excessive stuttering or large allele dropout. Deviations from linkage equilibrium were tested in GENEPOP (Rousset 2008).

3. Results

All 12 loci were used in both microsatellite multiplexes were polymorphic, with an average of 12 alleles per locus (ranging from 3 to 29 alleles per locus) and H_O ranging from 0.368 to 0.921. Significant deviations from Hardy–Weinberg equilibrium were observed at locus Par7 in multiplex 1 and loci Fwc08 and Fwc17 in multiplex 2. No evidence of linkage disequilibrium was observed. MICROCHECKER detected evidence for null alleles only for locus Par7 and no evidence of scoring errors due to stutter or large allele dropout were detected. Therefore, these two microsatellite multiplexes will be a valuable resource for ongoing and future studies of conservation genetics in the Caribbean spiny lobster, *Panulirus argus*.

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