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Genetic markers in blue crabs (*Callinectes sapidus*) I: Isolation and characterization of microsatellite markers

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Abstract

Given the commercial and ecological importance of the dwindling Chesapeake Bay blue crab (*Callinectes sapidus*) fishery, there is a surprising scarcity of information concerning the molecular ecology of this species. The few studies published to date are based on allozyme data and indicate a single, panmictic population along the Atlantic coast. To address this shortcoming, we have initiated the development of genetic markers from both the nuclear and mitochondrial genomes of the blue crab. To this end, we performed two separate screenings of the blue crab nuclear genome using both dinucleotide and tetranucleotide repeat oligonucleotide probes and the highly efficient "FIASCO" (Fast Isolation by AFLP of Sequences COntaining repeats) methodology. Our screenings produced 34 microsatellite loci. Genotyping of a captive-mated pair of blue crabs and 30 of their offspring at 10 of our isolated loci shows that eight loci are inherited in true Mendelian fashion, with two loci being monomorphic. Additionally, we genotyped 102 blue crab DNA samples collected from different parts of the Chesapeake Bay with the same 10 loci. The results of these screenings, including heterozygosities ranging between 0.26 and 0.97, indicate that a majority of the loci isolated in our screen will ultimately be useful markers for population genetic studies. The molecular tools described in this paper will be used, in tandem with differences in the blue crab mitochondrial genome [Place, A.R., Feng, X., Steven, C.R., Fourcade, H.M., Boore, J.L., 2005. Genetic markers in blue crabs (*Callinectes sapidus*) II: Complete mitochondrial DNA sequence and characterization of variation. J. Exp. Mar. Biol. Ecol. 319, 15–27], to investigate potential genetic substructure within the Chesapeake Bay and across the entire Atlantic Coast/Gulf Coast range of the blue crab, as well as monitor the results of restocking hatchery-reared crabs into the Bay.

Keywords: Allele frequency; Blue crab; *Callinectes sapidus*; FIASCO; Microsatellite; PCR

Abbreviations: 6-FAM, 6-carboxyfluorescein; AFLP, amplified fragment length polymorphism; ARC-II, Aquaculture Research Center II; COMB, Center of Marine Biotechnology; DNA, deoxyribonucleic acid; dNTP, deoxynucleotide triphosphate; EDTA, ethylenediaminetetraacetic acid; FIASCO, fast isolation by AFLP of sequences containing repeats; HEX, 6-carboxy-2′,4,4′,5′,7,7′-hexachlorofluorescein; MIH, molt-inhibiting hormone; MIH-SSR, molt-inhibiting hormone simple sequence repeat; mtDNA, mitochondrial deoxyribonucleic acid; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; SSR, simple sequence repeat; VNTR, variable number of tandem repeats.

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1. Introduction

The blue crab (*Callinectes sapidus*) has historically supported one of the largest and most successful commercial and recreational fisheries in the Chesapeake Bay (Cronin, 1998; Rugolo et al., 1998). In recent years, record-low catches of blue crabs have prompted renewed interest in its biological and ecological characteristics. Genetic analysis of population structure in the blue crab has received surprisingly little attention, in view of the commercial and ecological importance of the species. Early studies of protein polymorphisms (allozymes) suggested similar gene frequencies in Chesapeake Bay and Chincoteague Bay populations, but differences between these populations and South Carolina (reviewed in Burton and Feldman, 1982). Heterozygote deficiencies in the Chesapeake Bay and Chincoteague Bay populations reported by Cole and Morgan (1978) may reflect the mixing of genetically differentiated subpopulations, but may also have been a technical artifact resulting from the mis-scoring of gels.

McMillen-Jackson et al. (1994) presented a more thorough analysis of geographic variation in the blue crab, in which 750 individuals from 16 near-shore locations were scored for 31 presumptive loci. Significant heterogeneity in allelic frequencies was observed for several loci, but no large-scale geographic patterning was evident, with the exception of one locus (*EST-2*), which showed a latitudinal gradient in the Atlantic coast samples. Significant temporal variation (between-year and within-year) was also noted at this locus. Overall, the pattern reported by McMillen-Jackson et al. (1994) was one in which "allele frequencies varied significantly on range-wide and local scales, among and within collections, and among and within collecting years. Such a pattern is characteristic of genetic patchiness, which is a spatially and temporally chaotic distribution of heterogeneous allele frequencies in which local variation may be as great as long-distance variation" (Johnson and Black, 1982). The authors suggest that, in blue crabs, the observed patchiness may be the result of single-generation sampling effects, localized selection, or genetic drift.

Since the allozyme era, the advent of molecular techniques has revolutionized the field of population genetics. It is now possible to conduct population-level surveys using various types of DNA markers, which collectively provide a more robust and reliable picture of population structure in a given species (Lu et al., 2001; Strecker et al., 2003). We have chosen a two-pronged approach to addressing questions of the molecular ecology of the blue crab. The first approach involves identifying variable sequences in the maternally inherited mitochondrial genome (see Place et al., 2005). Our second approach is to identify and examine nuclear microsatellite loci. Microsatellite markers consist of variable numbers of tandemly repeated (VNTRs) simple sequences interspersed throughout the genomes of all organisms (Avise, 1994). Typically microsatellite loci are highly polymorphic and evolve rapidly, making them ideal genetic markers (Balloux and Lugon-Moulin, 2002; Schlötterer, 2000). Microsatellites are highly replicable and have a great ability to resolve genetic differences compared to other classes of genetic markers (Mueller and Wolfenbarger, 1999; Sunnucks, 2000). For these reasons, they are routinely used for forensic identification (Mukaida et al., 2000; Olaisen et al., 1997), pedigree analysis (Itokawa et al., 2003; McCouch et al., 1997), and population structure determination (Goldstein and Schlötterer, 1999; Schlötterer, 2000). We report here the identification of 34 microsatellite loci from the nuclear genome of the blue crab, as well as variation at a dinucleotide microsatellite locus located within the coding region of the gene encoding molt-inhibiting hormone (MIH; Lee et al., 1995).

2. Materials and methods

2.1. General

All DNA samples were prepared from either walking leg muscle (adults) or whole animals (juveniles) using the FastDNA Kit (QBIOgene, Carlsbad, CA).

2.2. Microsatellite loci isolation

Microsatellite loci were isolated from the genomic DNA of a single blue crab sampled from Chesapeake Bay in May 2002, using the method described in detail by Zane et al. (2002). Briefly, the ingenuity of