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## Utilization Of Sea Scallop (*Placopecten magellanicus* Gmelin) Microsatellite Markers For Phylogenetic Applications In Bay Scallops (*Argopecten irradians* Lamarck)

### Cover Page Footnote

We thank Lisa Campanella for her help in manuscript editing. This work was supported primarily by a Sokol grant for undergraduate research from Montclair State University. Liliana Kim graduated in Spring of 2006 with a BS from Montclair State University. Ms. Kim's primary advisor Dr. Campanella is a professor of Genetics at Montclair State University. He may be contacted by e-mail at [james.campanella@montclair.edu](mailto:james.campanella@montclair.edu) or phone at 973-655-4097. Review coordinated by Professor Andrea S. Cupp, Department of Animal Sciences, University of Nebraska-Lincoln.

## 1. INTRODUCTION

Bay scallops (*Argopecten irradians* Lamarck) are a common estuarine species along the eastern and southern coasts of North America from Massachusetts to Texas. Three clear subspecies (*A.i. irradians*, *A.i. concentricus*, *A.i. amplicostatus*) have been characterized within this geographic range based on shell morphometrics and molecular phylogenetics (Clarke 1965; Blake and Graves, 1995; Blake et al., 1997; Marelli et al., 1997; Bologna et al., 2001). The bay scallop is prized both commercially and recreationally throughout its range and represents an important species in both ecology and commerce.

The sea scallop (*Placopecten magellanicus* Gmelin) is a species that is closely related to the bay scallop. The sea scallop can be found in beds along the Atlantic coast of North America from Newfoundland to Virginia (Naidu 1991; Gjetvaj et al., 1997). As with bay scallops, sea scallops are commercially quite valuable as an aquacultured species in marine beds and laboratories. Molecular-based evidence indicates that *P. magellanicus* and *A. irradians* are genetically close, based on phylogenetic analyses of the 12S, 16S, and 18S rRNA genes (Steiner and Müller, 1996; Barucca et al., 2004). Steiner and Müller's (1996) study assigns sea and bay scallops to nearby branches of the same phylogenetic clade.

Several additional types of molecular discriminatory systems have been developed to characterize both sea and bay scallop populations. Allozyme (Beaumont and Zourous, 1991; Marelli et al., 1997), mitochondrial (Blake and Graves, 1995; Gjetvaj et al., 1992) and Random Amplified Polymorphic DNA (RAPD) (Patwary et al., 1994; Beaumont 2000) markers have all been developed for both species. Unfortunately, all these markers are inadequate for population studies due to various problems relating to data interpretation, laboratory application, inadequate genotype distinction, or limited availability of polymorphisms. For these reasons genomic microsatellite markers were developed for *P. magellanicus* (Gjetva et al., 1997). Genomic microsatellite loci do not have the limitations found in other markers. Regrettably, there are presently no published primer sequences to amplify genomic microsatellite markers in bay scallops. Due to this constraint, rapid and uncomplicated population studies are difficult to perform in *A. irradians*.

In other organisms (Blanquer-Maumont and Crouau-Roy, 1995; Morris et al., 1996; Colson et al., 1999; Paris et al., 2003; Campanella et al., 2004) interspecific microsatellite markers have been found to be effective tools for phylogenetic studies both within and between populations. We report here on sea scallop microsatellite DNA loci that are conserved in the bay scallop genome. This result lends additional support to the hypothesis that sea and bay scallops are closely related. There appears to be little alteration in the flanking sequences surrounding the seven microsatellite regions examined in *A. irradians*. Since, typically, flank-

ing primer regions for non-coding loci such as microsatellites are highly mutable and without selection pressure for conservation, the continued presence of these sequences may indicate that these two species are even more closely related than previously suggested. Additionally, these bay scallop microsatellite loci can be employed as powerful tools in characterizing the genetics and phylogenetics of natural and cultured populations of this species.

## 2. MATERIALS AND METHODS

### 2.1 Scallop Tissue Samples

Bay scallops (*Argopecten irradians* Lamarck) were collected within extensive eelgrass beds (July, 2004) in Little Egg Harbor, New Jersey, USA (39° 35'N, 74° 14'W), which is located in the central portion of the Mid-Atlantic Bight. Scallops were returned live to the laboratory and dissected. Adductor muscle tissue was extracted from individuals and stored frozen at -80°C for later DNA extraction.

### 2.2 DNA Extraction

Fifteen *Argopecten irradians* individuals were examined for the variation of microsatellites. The DNA was extracted from 0.5-0.8g of tissue. The tissue was stored frozen at -70°C and homogenized in an ice-cooled 1.5 ml microfuge tube and plastic DNase/RNase-free micropestle. The DNeasy extraction kit (Qiagen Corp., Valencia, CA, USA ) was used for DNA extraction following the manufacturer's directions.

### 2.3 Primers and PCR Amplification

The seven primer sets (Pma130, -132, -135, -180, -200, -212, and -275) developed for *Plocopecten magellanicus* by Gjetva et al. (1997) were synthesized for us by Invitrogen Corp (Carlsbad, California, USA). The PCR amplification conditions principally followed the directions of Gjetva et al. (1997). All PCR amplification was performed in a Mastercycler gradient thermocycler (Eppendorf, Inc., Westbury, NY, USA).

The annealing temperatures for primers Pma130, -132, and -212 had to be increased by 2-4°C above the original published values. Without the temperature increases employed for Pma130, -132, and -212 to obtain higher specificity, there was a great deal of background and secondary amplification.

The Pma275 and Pma135 loci would not amplify at all until the annealing temperatures were decreased by  $\sim 3^{\circ}\text{C}$  and  $\sim 5^{\circ}\text{C}$ , respectively (Table 1). The large decrease needed to allow amplification of the Pma135 polymorphism suggests that it may be the least conserved of the microsatellite loci.

**Table 1.** Optimal primer annealing temperatures for *A. irradians* and amplified allele sizes

Locus	Primer Annealing Temp.	Allele sizes
Pma130	53.5°C	118,153,166,176
Pma132	51.7°C	182,222
Pma135	51.9°C	87,125,166,200,238,275
Pma180	50.0°C	115,162,200,250
Pma200	48.4°C	178,310,380
Pma212	53.0°C	438,500,520,890
Pma275	48.1°C	174,200,248,292

Pma180 and Pma200 were amplified in bay scallop using the published temperatures (Gjetva et al. 1997). We increased the annealing temperatures for these two alleles to boost specificity, but we did not obtain fewer alleles. As the temperature increased, we eventually lost all annealing of primers and obtained no products.

#### 2.4 Microsatellite Fragment Analysis

DNA fragments were electrophoretically separated and analyzed by the method of Todokoro et al. (1995). The PCR products were imaged with Scion computer software (Scion, Inc., Frederick, MD, USA). Molecular weights were analyzed and calculated with Collage Version 4.0 (Image Dynamics Corporation, Surrey, BC, Canada).

### 3. RESULTS

All seven available *P. magellanicus* primers (Pma130, -132, -135, -180, -200, -212, and -275) amplified employing *A. irradians* as the genomic DNA template (Table 1). We found that only two of the loci tested (Pma180 and Pma200) amplified in bay scallop DNA with annealing temperatures that were essentially unchanged from those used originally with sea scallops (Gjetvaj et al., 1997).

The Barnegat population of *A. irradians* examined in this study possessed relatively little allelic variation (2-6 alleles) compared to the sea scallop population examined by Gjetvaj et al. (1997). Sea scallops evinced between 6 and 24 alleles for these same markers. Despite this “reduced” level of polymorphic variation found in the Barnegat Bay *A. irradians* population, it is evident that the seven intraspecific loci described have enough discriminatory power to potentially distinguish between different bay scallop populations.

All of the markers examined in bay scallops demonstrated evidence of polymorphisms (Tables 2 and 3). The loci displayed various levels of allelic variation, along with the presence of assorted heterozygote alleles and homozygotes. The Pma135 and Pma212 loci had the greatest number of different alleles concurrent with a high level of heterozygosity (Tables 2 and 3). These loci are possible genomic hot spots for mutation. This conclusion is strengthened by the results of Gjetvaj et al. (1997) which also show a relatively large number of heterozygous alleles with high variation at Pma135 and Pma212 in sea scallop. The Pma132 locus also demonstrated a high level of heterozygosity but manifested a very limited number of heterologous alleles.

### 4. DISCUSSION

We have described here alternative applications for seven polymorphic loci that were originally characterized in the sea scallop (Gjetvaj et al., 1997). We propose employment of these microsatellites as a genetic tool to help examine populations of the important aquacultured organism bay scallop. We have found that all the sea scallop markers tested can be PCR amplified from bay scallop DNA with a relatively high level of heterozygosity and polymorphic variation. These markers should prove to be a valuable phylogenetic tool for future examination of *A. irradians* populations.

**Table 2.** Representative microsatellite genotypes for Barnegat Bay *A. irradians* population.

Individual	Pma130	Pma132	Pma135	Pma180	Pma200	Pma212	Pma275
1	118/118	182/222	87/275	162/250	178/310	438/890	174/200
2	118/118	182/222	87/238	162/250	178/310	500/890	174/174
3	118/153	182/222	125/125	162/200	178/310	890/890	174/248
4	118/166	182/222	n/a	162/162	178/310	438/890	174/174
5	118/176	182/222	125/166	162/162	178/310	500/890	174/248
6	118/118	182/222	125/125	115/162	178/380	500/890	174/174
7	118/118	182/222	125/125	162/162	178/310	500/890	174/292
8	118/153	182/222	125/166	162/162	178/178	438/890	174/174
9	118/118	182/222	125/125	162/162	178/310	500/890	174/174
10	118/118	182/222	125/125	162/162	178/310	500/890	174/222
11	118/118	182/222	125/125	162/162	178/310	500/890	174/174
12	118/153	182/222	125/166	162/162	178/310	520/890	174/174
13	118/118	182,222	125/125	162/162	178/310	438/890	174/200
14	118/153	182/182	166/200	162/162	178/310	438/890	174/174
15	118/118	182/182	87/87	162/162	178/310	438/438	174/174

n/a= not amplified

**Table 3.** Microsatellite variation in a population of *A. irradians*.

Locus	Sample Size	Allelic range(bp)	# of alleles	observed heterozygotes	Est. Exp. Het.
Pma130	15	118-166	4	6	0.4490
Pma132	15	110-182	2	13	0.6906
Pma135	14	87-275	6	6	0.6800
Pma180	15	115-250	4	4	0.3601
Pma200	15	178-380	3	14	0.5303
Pma212	15	438-890	4	13	0.6283
Pma275	15	174-292	4	6	0.4671

Estimated Expected Heterozygosity calculated by Microsat 2.0 (Minch et al., 1995)

There were no homozygous null alleles observed in bay scallops for any of the sea scallop loci tested. Previous ribosomal-based molecular evidence indicated that *P. magellanicus* and *A. irradians* are genetically very close kindred and dubbed “sister groups” (Steiner and Müller, 1996; Barucca et al., 2004). Our results support this close relationship between the two species, and despite the high levels of DNA modification leading to polymorphic changes in the various alleles examined, there is a high degree of conservation for the flanking regions around these alleles-- even across the *Placopecten* and *Argopecten* genus border.

However, two caveats should be noted. First, the chance of primers annealing to multiple regions increases as annealing temperatures fall to below 53°C. Second, it is possible that the markers that span such a broad size range may not be amplifying in the same region of the genome (Tables 2,3). Although founding alleles sometimes result in the establishment of populations with wide ranges in allele sizes, mistakes in DNA replication tend to be incremental (Elfstrom et al. 2005).

Scientists have determined Previous data indicates Previous data (Gjetvaj et al., 1997) indicates that the number of alleles in the sea scallop population was much higher (6-24 alleles) than those observed in the Barnegat Bay scallop population (2-6 alleles)(Tables 1, 2, 3). One explanation for this phenomenon may be that a more isolated broadcast spawning species, such as the bay

scallop, would have a reduced level of outside gene flow than a coastal marine species such as *P. magellanicus*. The sea scallop population examined in the Gjetvaj et al., (1997) study was probably not genetically isolated and therefore prone to high gene flow from mixing of pelagic larval sources and influx of external individuals from mobile adults from nearby populations. This observation reinforces the existence of greater genetic isolation in estuarine species, but also indicates how particularly useful these polymorphic markers may be to bay scallop population studies.

Future investigations into stock assessment and enhancement can use these markers to assess population structure and heterozygosity as well as determine the success of enhancement programs through use of novel genetic markers.

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