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James Campanella
Montclair State University, campanellj@mail.montclair.edu

Paul Bologna
Montclair State University, bolognap@montclair.edu

L. E J Kim
Montclair State University

John V. Smalley
Bergen Community College

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MOLECULAR GENETIC EVIDENCE SUGGESTS LONG ISLAND AS THE GEOGRAPHIC ORIGIN FOR THE PRESENT POPULATION OF BAY SCALLOPS IN BARNEGAT BAY, NEW JERSEY

JAMES J. CAMPANELLA,[1]* PAUL A. X. BOLOGNA,[1] LILIANA E. J. KIM[1] AND JOHN V. SMALLEY[2]

[1]*Montclair State University, Department of Biology and Molecular Biology, 1 Normal Avenue, Montclair, New Jersey 07043;* [2]*Bergen Community College, Department of Science and Technology, 400 Paramus Road, Paramus, New Jersey 07652*

ABSTRACT We have used molecular genetic methods to examine the question of the geographic origins of the newly returned *Argopecten irradians* populations in Barnegat Bay (BB), New Jersey. Using PCR to amplify specific polymorphic microsatellite regions for bay scallop, we have genetically compared the 2004 and 2005 BB populations to those from Long Island (LI), New York, and North Carolina (NC). Our studies indicate that the 2004 and 2005 BB populations are genetically similar with some allelic frequency differences. Five of the eight loci studied are identical for marker size among BB, LI, and NC populations. The S336 locus demonstrates polymorphic sequences of 138 and 158 basepairs in the NC population that are not observed in LI or BB. The C1832 locus appears identical (122 basepairs) between LI and BB, but demonstrates polymorphisms (132 or 142 basepairs) in the NC population. Additionally, the NC group manifests two further alleles in the M26 locus (135 and 149 basepairs) not seen in BB or LI. These results, along with genetic distance and mean estimated gene flow calculations, support a physical transfer of the Long Island bay scallop larvae down the Atlantic coast to the transition regions around Barnegat Bay.

KEY WORDS: bay scallops, *Argopecten irradians*, microsatellite markers, DNA fingerprinting, sequence length polymorphisms, phylogenetic analysis

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 & 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 ecology and commerce.

Historically, bay scallops were abundant and commercially fished in New Jersey, USA. The first available landing records were collected in 1956, when 52,300 bushels were harvested with an estimated value of $287,000. Continued success of scallop populations for the next 12 y yielded 317,000 bushels valued at over $1 million (Ford 1997). Subsequently, commercial bay scallop harvests were only recorded for 1973 and 1974.

Despite its local importance, little information exists on the ecology and population structure of New Jersey bay scallops, particularly because of the collapse of the commercial fishery. In recent years, it was generally believed that bay scallops no longer occurred in New Jersey waters. The observation of numerous scallops in 1998 in Little Egg Harbor, NJ (Bologna et al. 2001) prompted questions regarding the density and reproductive habits of this population, as well as its origin. New Jersey is believed to be the point of contact between the *A. i. irradians* and *A. i. concentricus* subspecies. Bologna et al. (2001) indicated that the scallops circa 1998 were genetically similar to those of North Carolina. Unfortunately, during the following four years (1999–2002) recurrent brown-tides occurred in Barnegat Bay (Gastrich et al. 2004) and the scallop population declined. During the summer of 2000, a population survey was conducted in Barnegat Bay. Results of this survey showed the presence of only two scallops collected during 129 scallop dredge tows and no spat were collected (Bologna unpubl. data). Subsequent to this research, no scallops were observed in the bay between 2001 and 2003 (Bologna pers. obs.). However, in 2004 bay scallops were once again seen in Barnegat Bay (densities $\sim$0.1 m^{-2}) and a successful spat fall was recorded in August 2004 and 2005 (Bologna unpubl. data). The return of scallops in Barnegat Bay, NJ posits the question of larval origin for the recolonization.

It was the goal of this present research to (a) investigate the genetic population structure of the these newly arisen Barnegat Bay (BB) scallops from 2004 and 2005 using species-specific microsatellite loci (Roberts et al. 2005) and (b) compare allelic frequencies among the Barnegat Bay, Long Island, NY (LI), and North Carolina (NC) bay scallop populations with the intent of establishing the geographic origin of the current BB population.

MATERIALS AND METHODS

Scallop Tissue Samples

Bay scallops (*Argopecten irradians* Lamarck) were collected within extensive eelgrass beds (July 2004 and August, 2005) in Little Egg Harbor, NJ, USA (39°35′N, 74°14′W), which is located in the central portion of the MidAtlantic Bight. The Long Island scallops were collected in 2005 by Dr. Brad Peterson (Long Island University) from Peconic Bay. North Carolina scallops were collected in 2005 by Wayne Cuthrell and Marc Hamric (North Carolina Division of Marine Fisheries) in Bogue Sound off the coast of Morehead City, NC.

Scallops were returned live to our laboratory and dissected. Adductor muscle tissue was extracted from individuals and

*Corresponding author. E-mail: james.campanella@montclair.edu

stored frozen at –80°C for later DNA extraction. Long Island and North Carolina samples were shipped overnight on ice to New Jersey.

DNA Extraction

DNA was extracted from fifteen 2004 and thirteen 2005 Barnegat Bay scallops. Twenty Long Island and 20 North Carolina scallops were also used. The DNA was obtained from 0.5–0.8 g of adductor muscle tissue. The tissue was stored frozen at –80°C and homogenized in an ice-cooled 1.5 mL microfuge tube with a plastic DNase/RNase-free micropestle. The DNeasy extraction kit (Qiagen Corp., Valencia, CA) was used for DNA extraction following the manufacturer's directions.

Primers and PCR Amplification

Eight microsatellite primer sets were developed for bay scallops by Roberts et al. (2005). These primers were synthesized for us by Invitrogen Corp (Carlsbad, CA). The PCR amplification conditions generally followed the directions of Roberts et al. (2005). The only protocol alteration was an increase in the annealing temperature to 60°C used with the GP63 locus to reduce the emergence of triplets. All PCR amplification was performed in a Mastercycler gradient thermocycler (Eppendorf, Inc., Westbury, NY).

Microsatellite Fragment Analysis

DNA fragments were electrophoretically separated in a high-resolution manner and analyzed by the methods used in our previous studies (Vander Zwan et al. 2000, Campanella et al. 2003, Provan & Campanella 2003, Campanella et al. 2004). The PCR products were imaged with Scion computer software (Scion, Inc., Frederick, MD). Molecular weights were analyzed and calculated with Collage Version 4.0 (Image Dynamics Corporation, Surrey, BC, Canada).

Population Genetic Calculations

Chord distances (Cavalli-Sforza & Edwards 1967) were computed using the Microsat program (Minch et al. 1995). The neighbor-joining radial tree was calculated employing the chord distance data into the Phylip subroutine Neighbor (Felsenstein 1993), and the tree itself was generated by Treeview (Page 1996). The mean estimated gene flow (Nm) (Slatkin & Barton 1989) was determined using Popgene v1.32 (Yeh et al. 1997, Yeh & Boyle 1997).

RESULTS

The 2004 and 2005 Barnegat Bay populations show no differences in molecular weights in all eight microsatellite markers observed (Table 1), although allele frequency does differ between the two populations. We used a chord distance calculation (Cavalli-Sforza & Edwards 1967), which makes no model assumptions for the genetic distance estimate (Table 2). The chord distances indicate the genetic separation between the two BB populations and reflect the differences in allelic frequency.

Although allele frequency differed among the four scallop populations, five of the eight loci studied are identical for marker size among BB, LI, and NC (Table 1). The S336 locus evidences polymorphic fragments of 138 and 158 basepairs in the NC population that are not observed in the LI or BB populations. The C1832 locus appears identical (122 bp) between LI and BB, but demonstrates polymorphisms (132 or 142 bp) in the NC population not present in BB or LI. Additionally, the NC group manifests two further alleles at the M26 locus (135 and 149 bp) not seen in BB or LI (Table 1).

The genetic distances were used to generate a radial, neighbor-joining tree (Fig. 1). The North Carolina population is positioned at the end of a long branch, whereas LI falls into a clade with the 2004/2005 Barnegat Bay groups, indicating a closer relationship among the latter cluster.

The calculated genetic distance and neighbor-joining tree both support the hypothesis of a physical transfer of the Long Island bay scallop larvae down the Atlantic coast to the Atlantic Bight transition regions around Barnegat Bay.

TABLE 1.

Microsatellite alleles and polymorphisms examined in the bay scallop populations.

Marker	BB 2004(15)	BB 2005(13)	LI(20)	NC(20)
S336	128/168*	128/168	128/168	128/**138**/**158**/168
G340	114/129	114/129	114/129	114/129
C1831	122/142	122/142	122	122/142
C1832	122	122	122	**132/142**
GP63	200/223/285	200/223/285	200/223/285	200/223
N391	243/292	243/292	243/292	243/292
GL23	132	132	132	132
M26	120/157	120/157	120/157	120/**135**/**149**/157

* Molecular weights are in basepairs, the value in parenthesis = N, polymorphisms in bold are not found in BB or LI populations, and the "/" indicates heterologous allele sizes.

DISCUSSION

Several molecular discriminatory systems have been developed to characterize bay scallop populations in the last several years, including: allozyme (Beaumont & Zouros 1991, Marelli et al. 1997, Bologna et al. 2001), mitochondrial (Blake & Graves 1995, Gjetvaj et al. 1992) and Random Amplified Polymorphic DNA (RAPD) (Beaumont 2000) markers. Unfortunately, all of these markers are inadequate for population studies because of problems relating to data interpretation, laboratory use, inadequate genotype distinction, or limited availability of

TABLE 2.

Chord Distance Matrix indicating the genetic distance between the various bay scallop populations.

	BB 2004	BB 2005	LI	NC
BB 2004	0.000			
BB 2005	0.095	0.000		
LI	0.129	0.108	0.000	
NC	0.281	0.266	0.297	0.000

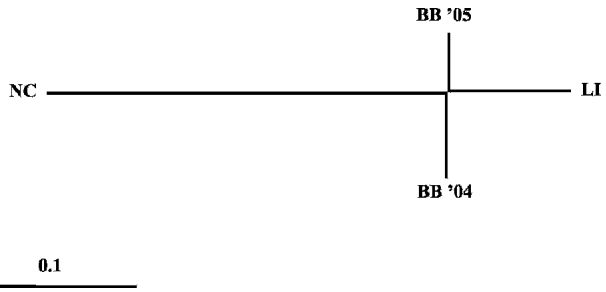

Figure 1. Unrooted, radial neighbor-joining tree of the four bay scallop populations. The chord distance data was analyzed by the Phylip subroutine Neighbor (Felsenstein 1993), and the tree was generated by Treeview (Page 1996).

polymorphisms. Roberts et al. (2005) microsatellite loci have none of these weaknesses and offer clear, easily scored polymorphisms that are quickly and directly obtained. We have taken advantage of these new markers to examine the phylogeographic origins of the recently discovered populations of bay scallops in New Jersey.

We have found population genetic evidence that bay scallops previously extant in New Jersey waters were most recently recruited from New York and not North Carolina. Bologna et al. (2001) established that the BB scallops sampled in 1998 were genetically similar to those of North Carolina. We hypothesize that this apparent discrepancy with our present results can be resolved by invoking a "subspecies shift" having occurred in Barnegat Bay. The previous New Jersey population appears to have been completely eradicated between 2000 and 2002 by brown tides. Brown tide has been shown to negatively impact the physiology of bivalves by reducing their feeding and respiration rates (Gainey & Shumway 1991, Bricelj & Lonsdale 1997). As a result, gonadal mass does not increase for reproduction, and the potential for population collapse increases. During the 1999–2002 brown-tide events, scallop density decreased and the species was absent from sampling regimes in 2001–2003 (Bologna unpubl. data). Our data support that the replacement population did not originate from the South, but was colonized instead from the Long Island, NY population. New Jersey resides at the midAtlantic Bight in a marine transition zone, the perfect location to be potentially affected by species transfer from either direction.

To invoke a subspecies replacement, an assessment of the potential larval transport mechanism is essential. Sverdrup et al. (1942) identified the southerly coastal currents along the Mid-Atlantic region of the United States, which provides the net transport of water masses from New York to New Jersey. Additionally, the potential tidal and Eckman transport into the coastal bays adjacent to the current provide a plausible mechanism for larval delivery into Barnegat Bay (see Epifanio & Garvine 2001). As such, the most germane possibility is transport from New York to New Jersey and this is demonstrated in our results.

This extinction/replacement scenario brings up the general question of the stability of populations. How stable is a marine population living in a very dynamic environment? It is possible that the New Jersey bay scallop populations have gone extinct and been replaced many times by various source populations, because it appears to have happened at least once. We have no information on whether this cycle has occurred before in this instance, but this phenomenon has been observed for other populations (Keough & Chernoff 1987, Tettelbach & Wenczel 1993, Colson & Hughes 2004). However, the results from Bologna et al. (2001) showing a close relationship with North Carolina populations, coupled with the southerly coastal currents (Epifanio & Garvine 2001), suggests that this is the first occurrence of this phenomena in *A. irradians* from New Jersey.

TABLE 3.

The mean estimated gene flow (Nm) matrix indicating the Nm values between the various bay scallop populations.

	BB 2004	BB 2005	LI
BB 2004			
BB 2005	18.2350		
LI	4.6550	4.8760	
NC	1.0200	1.1258	0.8760

Further evidence supporting this hypothesis is obtained from our mean estimated gene flow (Nm) analysis. We calculated the Nm values (Slatkin & Barton 1989) between the various populations and found that gene flow is much higher between the BB populations and LI than between BB and NC (Table 3). Although we are employing limited population sample sizes, these Nm values may indicate a higher degree of genetic interaction between the LI and BB populations. An internal control for this conclusion can be seen in the expectedly small gene flow value between North Carolina and Long Island (Table 3). This small estimate is consistent with the results of Blake and Graves (1995) who used mitochondrial DNA polymorphisms to examine geographically isolated populations along the Eastern Seaboard.

Understanding the basic population structure of any commercially exploited species is important for determining the viability of these stocks as well as the potential for interbreeding among stocks. Currently, there is insufficient genetic information regarding bay scallops. The population information that we have gathered here will be essential for fisheries enhancement and aquaculture (e.g., stock selection), but it will also provide a foundational assessment of New Jersey bay scallops. Given the potential of recurrent brown-tides causing broad scale extirpation in this species, the results of this research will be broadly applicable to scientists, fisheries managers, and commercial harvesters.

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