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MITOCHONDRIAL DNA VARIATION IN THE BAY SCALLOP, *ARGOPECTEN IRRADIANS* (LAMARCK, 1819), AND THE ATLANTIC CALICO SCALLOP, *ARGOPECTEN GIBBUS* (LINNAEUS, 1758)

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ABSTRACT Restriction site variation of the mitochondrial DNA of *Argopecten irradians* ssp. was surveyed within four populations from the U.S. Atlantic coast and the Gulf of Mexico. A population from North Carolina was resampled one year after the first collection to provide a measure of temporal variation within a population. Haplotype diversity was high, with 49 haplotypes revealed among a total of 135 bay scallops screened with 8 restriction endonucleases. Nucleotide sequence divergences corrected for within-sample variation among populations ranged from 0.00%, between the temporal samples, to 0.33% between geographically distant populations. Tests for heterogeneity indicated that no two of the geographically separated populations shared a common gene pool. UPGMA cluster analysis based on nucleotide sequence divergences suggested that bay scallops in North Carolina waters are more closely related to those from New England than to those from the Florida Gulf, though only slightly.

Two populations of the calico scallop, *Argopecten gibbus* Dall, from the Atlantic and Gulf coasts of Florida, were sampled and analyzed similarly. In these, haplotype diversity was high, with 6 restriction endonucleases revealing 19 different haplotypes among 51 individuals. Nucleotide sequence divergence between the geographically distant populations was low, however, and a test for heterogeneity was consistent with the null hypothesis of a shared, common gene pool for the two populations. No restriction fragment patterns were common to the bay and calico scallop, reinforcing their classification as separate species.

KEY WORDS: Bay scallop, calico scallop, *Argopecten*, mtDNA variation, geographic variation, restriction fragment length polymorphism

INTRODUCTION

The genus *Argopecten* includes two species found in the Atlantic and Gulf coasts of the United States. *Argopecten irradians* Lamarck, the bay scallop, is a short-lived hermaphroditic species which grows rapidly and reaches reproductive maturity at a relatively young age. The calico scallop, *Argopecten gibbus* Dall, exhibits a life-history very similar to that of *A. irradians*, but dwells in a sandy-bottom offshore habitat rather than the shallow grass-bed regime of the bay scallop. Both species support economically significant fisheries (Shumway and Castagna 1994).

The bay scallop is distributed discontinuously along the Atlantic and Gulf coasts of the United States, between Cape Cod, MA and the Laguna Madre, TX. Frequently associated with eelgrass beds (*Zostera marina*), bay scallop populations are limited to estuarine and near-shore environments, with protected bays and sounds forming the primary habitat (Heffernan et al. 1988). Within its range, *A. irradians* has traditionally been divided into three subspecies whose geographic boundaries are not clearly delineated: the northern *A. i. irradians*, found from Cape Cod south to an area between New Jersey and Maryland; *A. i. concentricus*, found between New Jersey and North Carolina and on the Florida Gulf coast; and *A. i. amplicostatus*, whose range in the Gulf of Mexico extends south from Matagorda, TX and through the Laguna Madre (Clarke, 1965). In 1987, the existence of a fourth *A. irradians* subspecies, *A. i. taylorae*, was proposed by Petuch (1987). The holotype was collected in the Rabbit Key Basin of Florida Bay, and was distinguished from *A. i. concentricus* by its smaller and more fragile valves. The range of this subspecies is thought to be restricted to the dense, well developed *Thalassia* beds of Florida Bay and the waters west of the middle and upper Keys (Petuch 1987). The validity of this additional subspecies designation is complicated by the observation by Waller (1969) that there exist numerous museum samples of *A. i. concentricus* reportedly collected from the Florida Keys.

It has been suggested that populations designated *A. i. concentricus* in North Carolina are more closely related to those of the northern bay scallop subspecies than to the Gulf populations with the same taxonomic designation, and the unique subspecific designation for the Rabbit Key scallops has been questioned (Marelli et al., in press). The degree of intergradation between the four currently recognized subspecies, and the extent to which they are genetically distinct, are not known.

Whereas the range of the bay scallop spans most of the eastern coast of the U.S., that of the calico scallop, *A. gibbus*, is limited to more southern waters. The calico scallop is found on the Atlantic coast between Cape Hatteras, NC and Cape Canaveral, FL. It is also present in harvestable numbers on the northern Gulf coast of Florida, and has been reported to occur off of the West Indies (Sastry 1962). Although their different habitats may effectively isolate the two species, the range of *A. gibbus* overlaps considerably with that of the southern bay scallop subspecies, *A. i. concentricus*, most notably off Cape Hatteras and in the Gulf of Mexico. *A. gibbus* does differ morphologically from *A. i. concentricus*, but the high degree of variation and overlap in the distinguishing characters can complicate identification (Sastry 1962).

The recruitment dynamics of bay and calico scallops are not fully understood, and it is likely that different strategies apply to the two species. For both, the range of larval movement or transport that may occur during the approximately two-week pelagic stage can be quite extensive. Kirby-Smith (1970) suggested that juvenile calico scallops found off of the North Carolina coast may be recruited from populations spawning on the Atlantic coast of Florida. The basis for this suggestion was the observation that calico scallop juvenile abundances off of North Carolina are not

correlated with the spawning of adults in the same, highly transient populations. Hydrographic conditions may permit the sporadic recruitment of larvae generated in Florida spawnings to beds off of North Carolina, perhaps through entrainment in the Gulf Stream (Kirby-Smith 1970). This hypothesis is supported by the low levels of morphological and allozyme variation detected between calico scallop populations from the Marquesas Keys, FL, Cape Canaveral, FL and Cape Lookout, NC (Krause et al. 1994). This type of long distance dispersal is not likely to occur for the bay scallop, whose habitat is restricted to near-shore grass beds and the shelter of barrier islands. It is thought that most bay scallop larval recruitment depends on tidal circulation which acts to retain the planktonic larvae within the suitable habitat of a bay or estuary (Marshall 1960, Peterson and Summerson 1992). If the different recruitment strategies of bay and calico scallops are indeed as hypothesized, one might expect to see a fairly continuous pattern of genetic variation along the geographic range of the calico scallop, whereas the more geographically isolated bay scallop populations should show higher levels of differentiation.

In addition to the large body of ecological work with scallop species, extensive genetic studies have been conducted, using techniques of chromosomal analysis and manipulation, and protein electrophoresis (Beaumont and Zouros 1990). Analyses of scallop population structure and stock isolation have used allozyme data primarily, as summarized by Beaumont and Zouros (1990), and Beaumont (1991). Restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA has been widely applied in the field of marine population genetics (Ovenden, 1990), and the mitochondrial genomes of several members of the scallop family, Pectinidae, have been examined in some detail (Snyder et al. 1987, Gjetvaj et al. 1992). The family shows a broad range of mtDNA features, many very different from those of other metazoans. *Placopecten magellanicus,* the deep-sea scallop, exhibits the most dramatic divergence from the standard, with the largest known mtDNA molecule of any multicellular animal and a high degree of mtDNA size polymorphism within the species, attributed to varying copy numbers of a tandemly repeated sequence (Gjetvaj et al. 1992). No such sequences have been detected in the mtDNA of *A. irradians,* which has been found to possess a 16.2 kb molecule of invariant size. The mtDNA of the calico scallop, *A. gibbus,* has not been characterized by these techniques.

In this study we employed RFLP analysis of bay and calico scallop mtDNA to assess the levels of variation within and between populations of the same species, and between the two species. Specifically, we sought to evaluate the following null hypotheses: (1) geographically isolated populations of *A. irradians* share a common gene pool, (2) morphologically similar populations of *A. irradians* (i.e., those with the same subspecies designation) share a common gene pool, (3) mtDNA variation in a population of *A. irradians* is temporally stable, (4) individuals of the putative *A. i. taylorae* subspecies share a common gene pool with those of a sampled population of *A. i. concentricus,* (5) geographically isolated populations of *A. gibbus* share a common gene pool, and (6) *A. irradians* and *A. gibbus* share a common gene pool.

MATERIALS AND METHODS

Samples of 21 to 34 *A. irradians* were obtained from New England, North Carolina, Florida Bay, and the Florida Gulf coast (Table 1). These were assumed to approximate natural set in their

TABLE 1.

Bay and calico scallop sample descriptions.

Population	Code	Date of Collection	*n*
Bay Scallops			
Crystal River, FL	FL	9/92	27
New England	MA	9/92	26
Harker's Island, NC	NC	10/12/92	27
Harker's Island, NC	NCT	11/12/93	21
Rabbit Key, FL	RK	9/22/93	34
Calico Scallops			
Cape Canaveral, FL	CA	2/17/93	27
Apalachicola, FL	AP	4/14/93	24

sites of origin. The New England sample was hatchery-produced with broodstock from Martha's Vineyard, Buzzards Bay and Nantucket Sound. The broodstock had been maintained in the hatchery since 1988, and was used to produce seed scallops to augment the natural bay scallop set in the area. The sample was harvested from seeded beds in Nascatucket Bay, but may have also contained scallops from natural set. The hatchery had not introduced any scallops from outside New England into their broodstock at the time of collection (R. Taylor, Taylor Seafood, 1993, pers. comm.). The sample from the Florida Gulf coast was composed of laboratory-reared F_1 progeny of individuals harvested from Crystal River, FL. The broodstock from Crystal River had not been combined with any other stocks (N. Blake, University of South Florida, 1992, pers. comm.). North Carolina bay scallops were collected from natural set on the clam beds of Carolina Cultured Shellfish, Inc., off of Harker's Island, North Carolina. A temporal replicate of this sample was obtained one year after the first collection. Scallops from Florida Bay were collected from the fairly pristine, though sparsely populated natural beds off Rabbit Key.

Samples of *A. gibbus* comprising 27 and 24 individuals respectively, were obtained from the waters off Cape Canaveral, FL and Apalachicola, FL (Table 1). They represent natural set for these regions.

With the exception of the Rabbit Key bay scallops and the Apalachicola calico scallops, samples were delivered or hand-carried to the Virginia Institute of Marine Science (VIMS) alive, where gonad and/or mantle tissue were dissected and frozen at −70°C. Limited numbers of individuals were processed fresh when time permitted. In the exceptional cases, animals were shucked and frozen whole in liquid nitrogen, then stored at −70°C until transport to VIMS on dry ice.

Mitochondrial DNA was purified by cesium-chloride density-gradient ultracentrifugation from 2–3 g of gonad or mantle and gill tissue, using a modified homogenization buffer containing 200 mM sucrose (Lansman et al. 1981). MtDNA yield was observed to vary with the type of tissue used, with ripe gonad providing better yield than mantle and gill, or immature or spent gonad.

Digestion of 10–50 ng of *A. irradians* mtDNA was carried out with the following restriction endonucleases according to the manufacturer's recommendations: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, *Hin*dII. Many of the individuals in the *A. gibbus* sample from Apalachicola yielded low quantities of closed-circular mtDNA, perhaps because of an extended storage time, or inadequate handling procedures. Limited DNA quantities required that only six of the above enzymes be included in the calico scallop

analysis. These were *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, and *Hae*II. For comparison with *A. irradians*, Cape Canaveral calico scallop DNA was screened with all eight enzymes.

DNA from all *A. irradians* samples and the Cape Canaveral *A. gibbus* were visualized by endlabeling. Restriction fragments were endlabeled with the Klenow fragment of DNA polymerase I and ^{35}S-labeled nucleotides, electrophoresed at 1 volt/cm in 1% agarose gels overnight, and visualized by autoradiography (Sambrook et al. 1989). ^{35}S-labeled 1 kb ladder DNA (BRL) provided a molecular weight size standard.

The low yields of mtDNA from Apalachicola calico scallops made it necessary to visualize some restriction patterns by Southern transfer and hybridization (Sambrook et al. 1989). Digests of mtDNA or aliquots of mtDNA-enriched nuclear DNA were prepared as described previously, and were electrophoresed in 1% agarose gels at 3 volts/cm for 3–4 hours, with biotinylated λ/HindIII fragments as a size standard. Gels were blotted by overnight capillary transfer onto nylon membrane, and membranes were UV-crosslinked to immobilize the DNA fragments. Blots were prehybridized for 2 hr at 42°C (50% formamide, 5× SSC, 5× Denhardt's solution, 0.025 mM $NaPO_4$, pH 6.5, and 100 μg/mL heat-denatured calf thymus DNA). Overnight (42°C) hybridization was initiated with the addition of 0.25–0.5 μg biotinylated probe DNA prepared from purified *A. irradians*, *A. gibbus* or *Placopecten magellanicus* mtDNA by nick translation with biotin-7-dATP (BRL Bionick Labeling System). Following a series of post-hybridization washes (Sambrook et al. 1989) and blocking (3% bovine serum albumen), mtDNA fragments were visualized using the BRL BluGene Nonradioactive Nucleic Acid Detection System.

Sizes of mtDNA fragments were estimated by fitting band migration distances to those of the standard by the local reciprocal method of Elder and Southern (1983) using the program Gel Frag Sizer (Gilbert 1989). Restriction sites were inferred from completely additive fragment patterns, and letter designations were assigned to the different patterns. Six- or 8-letter composite haplotypes were compiled for the series of enzymes and analyzed separately for fragment and site data.

Statistical analyses were performed using the Restriction Enzyme Analysis Package (REAP) (McElroy et al. 1991). *A. irradians* and *A. gibbus* were analyzed separately, because they showed no similar restriction fragment patterns. For each sample, haplotype and nucleotide diversities were calculated following the methods of Nei (1987) and Nei and Miller (1990), respectively. Mean nucleotide sequence divergence between samples was calculated following Nei and Miller (1990), and was corrected for within-population polymorphism by subtracting the average of within-sample diversities. Since many of the haplotypes observed were rare, a Monte Carlo simulation (Roff and Bentzen 1989) was performed to estimate heterogeneity and assess the likelihood that the sampled populations shared a common gene pool.

TABLE 2.

***Argopecten irradians*. Composite haplotypes from seven populations.**

Haplotype	MA	NC	NCT	FL	RK	Total
AAAAAAAE	2					2
AABAAAAE	5					5
AABAAAAH	2					2
AACAAAAA	7	13	13	2		35
AACAAAAG	2					2
AACBAAAA	1					1
AADAAAEE	4					4
AAEAAAAE	2					2
AAFAAAEE	1					1
AACAAAAC		1				1
AACAAAAD		1				1
AACAAAGA		1				1
AACAABDD		1				1
AACABAAA		1				1
AACCAAAA		1				1
AAGAAAAE		1				1
ABCAAABA		1				1
ACCADAAA		1				1
ADCAAACA		1				1
AFCAAAAA		1				1
BACAAAAB		3		10		13
AEBAAAAA				2	3	5
AEBBAAAA				10		10
CABACAAA				2		2
AACAAAAI				1		1
AACAAAAE			1			1
AACAADAA			1			1
AACAAEAA			1			1
AACDAAAA			1			1
ACEAAAAE			1			1
AICAAAAA			1			1
DAIAAAAA			1			1
EACAAAAA			1			1
AABAAAAA					9	9
AABAAAAD					1	1
AABABAAJ					1	1
AABAEAAA					1	1
ACBAAAAA					1	1
ADBAAACA					1	1
AEBAAAAE					1	1
AEBAAAAH					1	1
AEBAAAEA					4	4
AEBAAAEK					1	1
AEBDAAAA					3	3
AGCAAAAA					1	1
AHHABAHA					1	1
DABABCAA					1	1
DCBABAAK					3	3
EEBDAAAA					1	1
Total *n*	26	27	21	27	34	135

MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; FL = Crystal River, FL; RK = Rabbit Key, FL. Restriction enzymes used: *Ava*I, *Ban*I, *Ban*II, *Bgl*II, *Bst*EII, *Eco*RI, *Hae*II, and *Hind*II.

RESULTS

A. irradians

Substantial genetic variation was observed within and among populations of the bay scallop. All enzymes revealed polymorphic restriction sites, producing between 4 and 10 distinct fragment patterns each. Analysis of 135 bay scallops with 8 restriction endonucleases revealed a total of 133 fragments and 49 distinct mtDNA haplotypes (Table 2). Individual enzymes revealed 6 to 16 restriction sites. Eighty-three sites were scored in total, and restriction site gains and losses were inferred from additive changes in fragment patterns. The most common composite haplotype, represented by 35 individuals, contained a total of 52 sites, accounting for approximately 1.7% of the mtDNA genome. The size

of the whole molecule was estimated at 16.7 kb from *Ava*I digests, and no indications of size polymorphism or heteroplasmy were apparent from these RFLP data.

Haplotype diversity, or the probability of encountering different haplotypes when two individuals are sampled from a population, was quite high (0.63–0.91) for all of the bay scallop samples (Table 3). Mean nucleotide sequence diversities ranged from 0.22%, for the second North Carolina sample (NCT), to a high of 0.53% for the Crystal River sample (FL). The high value for the latter population was not correlated with haplotype diversity, but was caused by the predominance of two divergent haplotypes in the sample, AEBBAAAA and BACAAAAB, that differed from each other by 5 sites. The first North Carolina (NC) sample contained 11 rare haplotypes, each represented by a single individual, but 9 of these differed from the common haplotype by only one or two site changes. Thus, the nucleotide sequence diversity within this population was comparatively low at 0.28%.

A matrix of mean nucleotide sequence divergences among the populations is presented in Table 4. Values below the diagonal are corrected for within-sample variation. Most notable among these is the corrected divergence between the two North Carolina samples (NC and NCT) collected at the same site in consecutive years. This value is negligible, indicating that the likelihood of sampling similar individuals from both populations is comparable to that of sampling them from within either population. The temporal samples were pooled for the determination of mean nucleotide sequence divergences between North Carolina and the other populations.

Heterogeneity analysis was performed on the pooled haplotype distributions from all of the bay scallop samples. One thousand Monte Carlo simulations yielded no χ^2 values exceeding the observed, indicating that the populations did not share a common gene pool. The two North Carolina populations were tested separately for heterogeneity using this method, with 987 simulations producing χ^2 values equal to or greater than the observed. This is consistent with the null hypothesis of a shared common gene pool, and as expected from the calculated divergence value, these two populations showed little temporal partitioning of variation. Tests for heterogeneity were likewise performed on geographically separate pairs of populations that shared a haplotype. In all of these, no χ^2 values higher than the observed were produced, indicating that significant heterogeneity exists between the populations.

Phenetic analysis by the unweighted pair-group method (UPGMA) performed on the mean nucleotide sequence divergences among samples of *A. irradians* illustrates the lack of differentiation between the two North Carolina samples (Fig. 1). These appear to be approximately equidistant from the cluster formed by the Rabbit Key (RK) and Crystal River (FL) samples, and the New England (MA) population.

TABLE 3.

***Argopecten irradians*. Summary statistics.**

	MA	NC	NCT	NC_{pool}	FL	RK
# individuals	26	27	21	48	27	34
# haplotypes	9	13	9	21	6	17
Haplotype diversity (*h*)	0.87	0.74	0.63	0.69	0.74	0.91
% mean nucleotide sequence diversity (*p*)	0.48	0.28	0.22	0.25	0.53	0.50

MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; NC_{pool} = NC + NCT; FL = Crystal River, FL; RK = Rabbit Key, FL.

TABLE 4.

***Argopecten irradians*. Matrix of nucleotide sequence of divergences among populations, in percent.**

	MA	NC	NCT	NC_{pool}	FL	RK
MA	—	0.51	0.46	0.49	0.68	0.61
NC	0.13	—	0.25		0.53	0.58
NCT	0.11	<0.01	—		0.51	0.54
NC_{pool}	0.12			—	0.52	0.56
FL	0.18	0.13	0.14	0.14	—	0.62
RK	0.12	0.19	0.18	0.19	0.11	—

Uncorrected above diagonal; below diagonal corrected for within-sample variation. MA = New England; NC = Harker's Island, NC; NCT = NC + 1 yr.; NC_{pool} = NC + NCT; FL = Crystal River, FL; RK = Rabbit Key, FL.

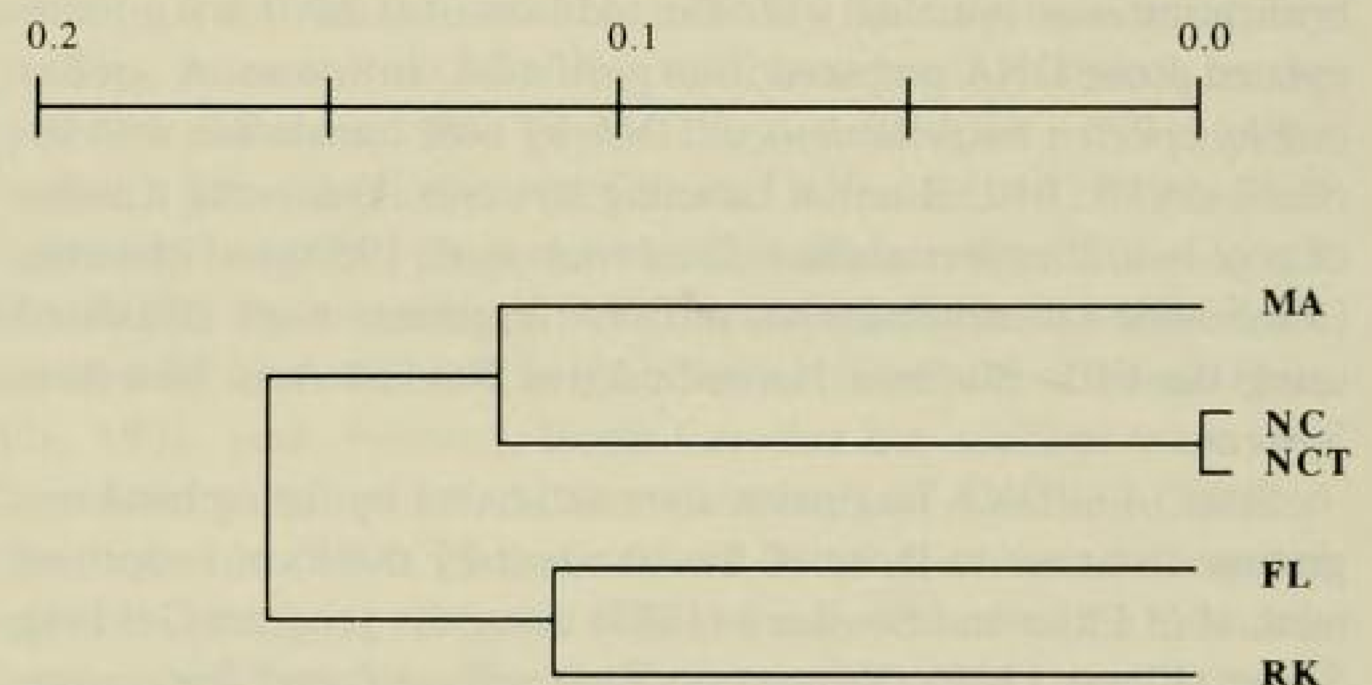

Figure 1. Dendrogram from UPGMA cluster analysis of five samples of *A. irradians*. Scale is percent nucleotide divergence. MA = New England; NC = Harker's Island, NC; NCT = NC + 1 year; FL = Crystal River, FL; RK = Rabbit Key, FL.

A. gibbus

In all, 51 calico scallops were screened with 6 restriction endonucleases, to produce 19 distinct mtDNA haplotypes (Table 5). Enzymes produced between 2 and 7 distinct fragment patterns, and none was invariant. A total of 60 fragments was visualized, and restriction site gains and losses were inferred from additive changes in fragment patterns. The different fragment patterns were accounted for with 3 to 12 sites, and 42 sites were scored in total. The size of the whole mtDNA molecule was estimated at 16.5 kb from the *Ava*I digests, and no indications of size polymorphism or heteroplasmy were apparent.

Five of the 19 haplotypes were shared between the two populations of calico scallops. The most common haplotype, aaaaaa, was present in both samples, in a total of 17 individuals. It comprised 36 restriction sites, accounting for an estimated 1.2% of the genome. The haplotype diversities of the Cape Canaveral and Apalachicola populations were both large at 0.79 and 0.92, respectively. Mean nucleotide sequence diversities were 0.54% for the Cape Canaveral sample, and 0.69% for the Apalachicola individuals (Table 6). Corrected and uncorrected nucleotide sequence divergences between the populations were 0.007% and

TABLE 5.

***Argopecten gibbus*. Composite haplotypes from two populations.**

Haplotype	CA	AP
aaaaaa	12	5
aaaaac	1	
aadaaa	1	
aaeaaa	3	
adaaaa	1	3
babaaa	4	4
babaaf	1	
bacaaa	1	1
bafaaa	1	1
bbbbaa	1	
bcbabb	1	
aaaaad		1
aaaaag		1
aaaace		1
adeaaa		2
adeaac		1
aegaaa		1
babaab		1
bdbaaa		2
Total *n*	27	24

CA = Cape Canaveral, FL; AP = Apalachicola, FL. Restriction enzymes used: *Ava*I, *Ban*I, *Bgl*II, *Bst*EII, *Eco*RI, and *Hae*II.

0.621% respectively, indicating that most of the sequence divergence could be attributed to variation within the samples.

A Monte Carlo test for heterogeneity performed for the two calico scallop samples yielded 143 out of 1000 replicates with χ^2 values exceeding that calculated from the original data, suggesting that the populations are not significantly heterogeneous. That is, the null hypothesis that the two populations share a common gene pool could not be rejected.

The Cape Canaveral sample of calico scallops was screened with the full battery of eight restriction enzymes used in the bay scallop analysis. No similar banding patterns or shared fragments were observed between the two species.

DISCUSSION

A. irradians

Significant genetic heterogeneity was revealed among the four geographically isolated bay scallop populations. This result is consistent with morphological differentiation among the populations (Clarke 1965). No clear trends were apparent in the magnitudes of corrected nucleotide sequence divergences and geographic distribution. The New England sample was equally divergent from the pooled North Carolina sample (0.12%) and the Rabbit Key sample (0.12%), but was slightly more divergent from the Florida Gulf population (0.18%). The pooled North Carolina sample, however, was more divergent from the Rabbit Key scallops (0.19%) than from the Florida Gulf sample (0.14%). This raises the question of the validity of assigning subspecies designations on the basis of these data. Since within-sample diversities were so high, it is difficult to determine trends among populations that might be indicative of relatedness at the subspecies level.

Pairwise testing of geographically separate populations with the same taxonomic designation did not reveal any that shared a common gene pool. Again this was likely due to the isolated nature of bay scallop populations, and the geographic distance separating the populations included in this study. The potential for sampling effects on these results should be considered, as error may have been introduced when scallops were collected. Removal of scallops from the field was performed by other researchers or culturists in all cases, and little is known about collection procedures used. It is assumed that the populations were effectively sampled with the removal of 30 to 50 individuals, but there remains the possibility that maternally-based patchy distributions were encountered within populations. Diversity values within populations were sufficiently high (0.63 to 0.91) that bias introduced by non-random sampling might be considered negligible. Interpopulation comparisons of diversity would not be valid, however, particularly because two of the "natural" samples were actually derived from cultured populations. The New England sample, as described earlier, had been subjected to broodstock introductions from a number of Massachusetts sources. The degree to which this population had been inbred is not known. The Crystal River sample was only one generation removed from the source population, but the measured diversity within this sample cannot be said to approximate that of Crystal River bay scallops. In the cases in which the samples were derived from populations which had been subjected to hatchery breeding, one might expect to see a significant decrease in genetic diversity due to inbreeding and the potential for a genetic bottleneck. Notably, neither the New England nor the Crystal River samples showed diversities below those of samples collected from naturally settled populations.

Nucleotide sequence divergence data based on an analysis of mtDNA were not inconsistent with the findings of a study of allozyme variation in bay scallops (Marelli et al., in press). Marelli et al. (in press) found individuals of a North Carolina population of *A. i. concentricus* to be genetically intermediate to New England *A. i. irradians* and Florida *A. i. concentricus*. Genetic-distance values (Nei's D) among the subspecies of *A. irradians* were found to range between 0.047 and 0.188, with increased divergences between populations separated by greater geographic distance. The genetic distance between bay scallops from western Florida and North Carolina was no smaller than that between the North Carolina population and a sample of *A. i. irradians* from Massachusetts (Marelli et al., in press).

UPGMA analysis based on mtDNA nucleotide sequence divergences placed North Carolina and New England bay scallops in a cluster separate from the Florida Gulf and Rabbit Key samples (Fig. 1). This agrees with an UPGMA dendrogram based on al-

TABLE 6.

***Argopecten gibbus*. Summary statistics.**

	CA	AP
# individuals	27	24
# haplotypes	11	13
haplotype diversity (*h*)	0.79	0.92
% mean nucleotide sequence diversity (*p*)	0.54	0.69

Nucleotide sequence divergence between CA and AP samples of *A. gibbus*, in percent:
Uncorrected: 0.621
Corrected for within-sample variation: 0.007

CA = Cape Canaveral, FL; AP = Apalachicola, FL.

lozymes (Marelli et al., in press), which also placed a North Carolina bay scallop population in a cluster with samples from Massachusetts, Connecticut and Long Island, while samples from the Florida Gulf and Rabbit Key clustered separately. Based on these dendrograms, neither the mtDNA nucleotide sequence divergence data nor the allozyme data support the preferred classification of North Carolina bay scallops as *A. i. concentricus*, as traditionally determined by morphological characteristics. Standard errors on the branches of these dendrograms make them essentially indistinguishable, however, and qualitatively both show North Carolina samples to be intermediate to two major clusters grouped closely along the lines of their morphologically-determined taxonomic designations. It is informative then, to consider bay scallops from New England and Florida's west coast to be separate subspecies, inasmuch as they are morphologically and genetically distinct, but it is not clear which of these classifications is most aptly assigned to the bay scallops of North Carolina. Based on morphology, North Carolina bay scallops should continue to be classified as *A. i. concentricus*, whereas genetically there is no evidence to suggest that this is a more appropriate designation than *A. i. irradians*.

Clarke (1965) delineated the range of *A. i. concentricus* to include the region between New Jersey and North Carolina. Unfortunately, it has not been possible to apply RFLP or allozyme analysis to bay scallop populations from this region, as none remain to be sampled. It seems probable that the clinal variation that places North Carolina bay scallops intermediate to those of New England and the Gulf, would also be apparent in a New Jersey sample, with the latter possessing morphological and genetic characters intermediate to those of New England and North Carolina.

The distributions of haplotypes among scallops sampled from the same North Carolina population in two consecutive years (i.e., two generations) were very similar, consistent with the hypothesis that the temporally isolated populations shared a common gene pool. Of the haplotypes observed in the NC and NCT samples, only one (AACAAAA) was common to both, but this was found in 48 and 62% of the individuals, respectively. This finding permits more confidence in the interpretation of results from geographically-separated populations. Since temporal variation was found to be relatively small, the high levels of variation within the geographically isolated samples may be taken as characteristic of their respective populations—that is, they are not just "noise" in the signal that might prevent characterization and distinction of populations with these genetic data. This finding also holds implications for the question of bay scallop larval dispersal ability. It is apparent from the lack of temporal variation in these samples that the scallops recruited to this population in the two years examined originated from the same or similar broodstock. One manner in which this temporal homogeneity may be maintained is by retention of larvae from a local spawning event, so that those recruited to the population are offspring of the previous generation. For a species that is short-lived and essentially annual, this may be used as a measure of recruitment success.

Heterogeneity analysis indicated that *A. i. taylorae* from Rabbit Key, Florida and the Crystal River population of *A. i. concentricus* did not share a common gene pool, although the Rabbit Key scallops clustered with the Crystal River sample in the UPGMA analysis. The nucleotide sequence divergence of 0.12% between the two populations would not seem to justify a separate subspecies status for the Rabbit Key scallops. These are at least as related to the Crystal River sample as is the sample designated *A. i. concentricus* from North Carolina. As such, the suggestion that *A. i. taylorae* was described from juvenile *A. i. concentricus*, is supported (Marelli et al., in press). Morphological variation between the populations may be limited to phenotypically plastic characters that are expressed differently in the two regions.

A. gibbus

Heterogeneity analysis of the calico scallop samples was consistent with the hypothesis of a shared, common gene pool for the two populations. This supports the suggestion that *A. gibbus* larvae are capable of wide dispersal (Kirby-Smith 1970, Krause et al. 1994). Entrainment in the Loop Current of the eastern Gulf of Mexico and/or the Gulf Stream might provide transport such that larvae from individuals spawned in the northern Gulf of Mexico could feasibly recruit to populations on the Atlantic coast of Florida. Transport in the opposite direction, from the Atlantic into the Gulf, does not seem as likely, but it is apparent that sufficient gene flow occurs to prevent divergence of these geographically separate populations by the mechanisms of natural selection and genetic drift.

Genetic divergences among populations of bay and calico scallops revealed few clear trends or biogeographic patterns, as have been observed for the oyster and horseshoe crab (Saunders et al. 1986, Reeb and Avise 1990). An UPGMA cluster analysis for the haplotypes observed in the calico scallop did not reveal two distinct phenetic groups characterizing the Atlantic and Gulf coast populations, as it did for *Crassostrea virginica* (Reeb and Avise 1990). If a break in the distribution of mtDNA haplotypes exists for the calico scallop, it may only be detectable at sites north of Cape Canaveral.

Clear differences in the mitochondrial DNA of bay and calico scallops were detected in the course of this study, indicating that RFLP analysis could provide a fairly simple diagnostic technique for distinguishing the two species. Both are marketed primarily as a shucked product—adductor muscle only—and in this form are virtually identical in appearance. RFLP analysis with a small battery of restriction enzymes should provide a useful tool for distinguishing the meats of the two species, as might be desirable for the regulation of the separate fisheries.

Perhaps the most interesting feature revealed in the comparative genetic analysis of the two species is the different patterns of geographic variation which correspond to differences in life history and recruitment dynamics. The calico scallop, which dwells offshore, and whose distribution is considered fairly continuous around the Florida peninsula, showed little genetic differentiation between two populations separated by considerable geographic distance. The bay scallop, conversely, showed heterogeneity among all of the populations examined. This reflects the relative isolation of bay scallop populations, which by virtue of limited habitat and a different recruitment strategy, maintain distinct haplotype distributions.

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