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GENETIC VARIATION IN THE CAROLINA MARSH CLAM, *POLYMESODA CAROLINIANA*

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ABSTRACT Horizontal starch gel electrophoresis was used to detect genetic variation at eight enzyme loci and five general protein loci in 11 populations of *Polymesoda caroliniana* from the Gulf of Mexico and south Atlantic coast of the U. S. Little variability was found between four of these populations along a salinity gradient in the Cape Fear Estuary, NC, and a regional trend was not observed in other populations along a latitudinal gradient. Heterogeneity analyses and dendrograms, both based on allele frequencies, suggest populations from the Gulf coast of Florida are genetically different from both a northern Gulf population (Mississippi) and Atlantic populations. The population from Mississippi was similar to populations from the Atlantic coast, all of which were similar. Heterozygosity in the 11 populations ranged between 8.11 and 28.0%, and the percentage of loci polymorphic between 37.5 and 71.4%. Populations conformed to Hardy-Weinberg expectations at greater than 95% of all loci assayed except glucose dehydrogenase, where only the populations from Fort Myers, FL, and Sapelo Island, GA, conformed to Hardy-Weinberg expectations. Electrophoretic patterns observed suggest *P. caroliniana* larvae are planktonic and effective at dispersal.

Introduction

The genetic structure of populations of marine invertebrates can be influenced by the mode of larval development (Crisp 1978, Liu et al. 1991, Hoskin 1997). Numerous investigators have demonstrated that species with planktonic larval stages show high levels of gene flow compared to species lacking such stages. For example, Hoskin (1997) investigated three gastropod species that have similar distributions in southeastern Australia: *Cominella lineolata* and *Bedeva hanleyi*, which undergo direct development in benthic egg capsules and emerge as crawling juveniles, and *Morula marginalba*, which produces planktonic larvae. *Cominella lineolata* and *B. hanleyi* exhibited high levels of variation among populations, whereas *M. marginalba* exhibited low levels of variation among populations. Janson (1987) also observed the same pattern of gene flow in brooding, i.e., egg carrying versus planktonic species of *Littorina*.

Polymesoda caroliniana, the Carolina marsh clam, is a member of the predominantly freshwater Corbiculidae and its reported range is from Virginia to Texas (Andrews and Cook 1951, Tabb and Moore 1971, Olsen 1973, Olsen 1976, Duobinis-Gray and Hackney 1982, Hackney 1983, Hackney 1985a). Cold intolerance may be the primary factor limiting its northern distribution (Hackney 1985b). It is found in a wide variety of shallow water or intertidal habitats, including salt marshes, brackish marshes, open river shores, mud banks, rock crevices and peat bogs, and is often associated with the plants *Spartina alterniflora*, *Juncus roemerianus*, *Taxodium distichum* and *Rhizophora mangle* (Andrews and Cook 1951, Tabb and Moore 1971, Olsen 1973, Olsen 1976, Duobinis-Gray and Hackney 1982, Hackney 1983, Hackney 1985a). *P. caroliniana* is considered euryhaline (Andrews and Cook 1951, Olsen 1973), occurring in habitats ranging from full strength seawater to fresh water, although it is more common and abundant in salinities less than 15‰ (Andrews and Cook 1951, Tabb and Moore 1971, Cain 1973, Hackney 1985a).

Little is known about the larval life history and dispersal mechanisms of *P. caroliniana*. Olsen (1976) spawned *P. caroliniana* in the laboratory with minimal success. He documented pelagic larval development of one individual to the straight-hinge stage, 66 h at a length of 78 µm, figures typical for other pelagic bivalve larvae. That recruitment of juvenile *P. caroliniana* does not always coincide with spawning events suggests a potential for long-term residence as meroplankton (Hackney 1983). To determine more about the larval life history and dispersal mechanisms of *P. caroliniana*, this study analyzed genetic (enzymatic) variation in 11 populations from the Gulf of Mexico and south Atlantic coast, including four along a salinity gradient in one estuary, and seven from different parts of the species' range.

Materials and Methods

Site descriptions

Samples of eleven populations were collected from estuaries along the Atlantic and Gulf coasts, from North

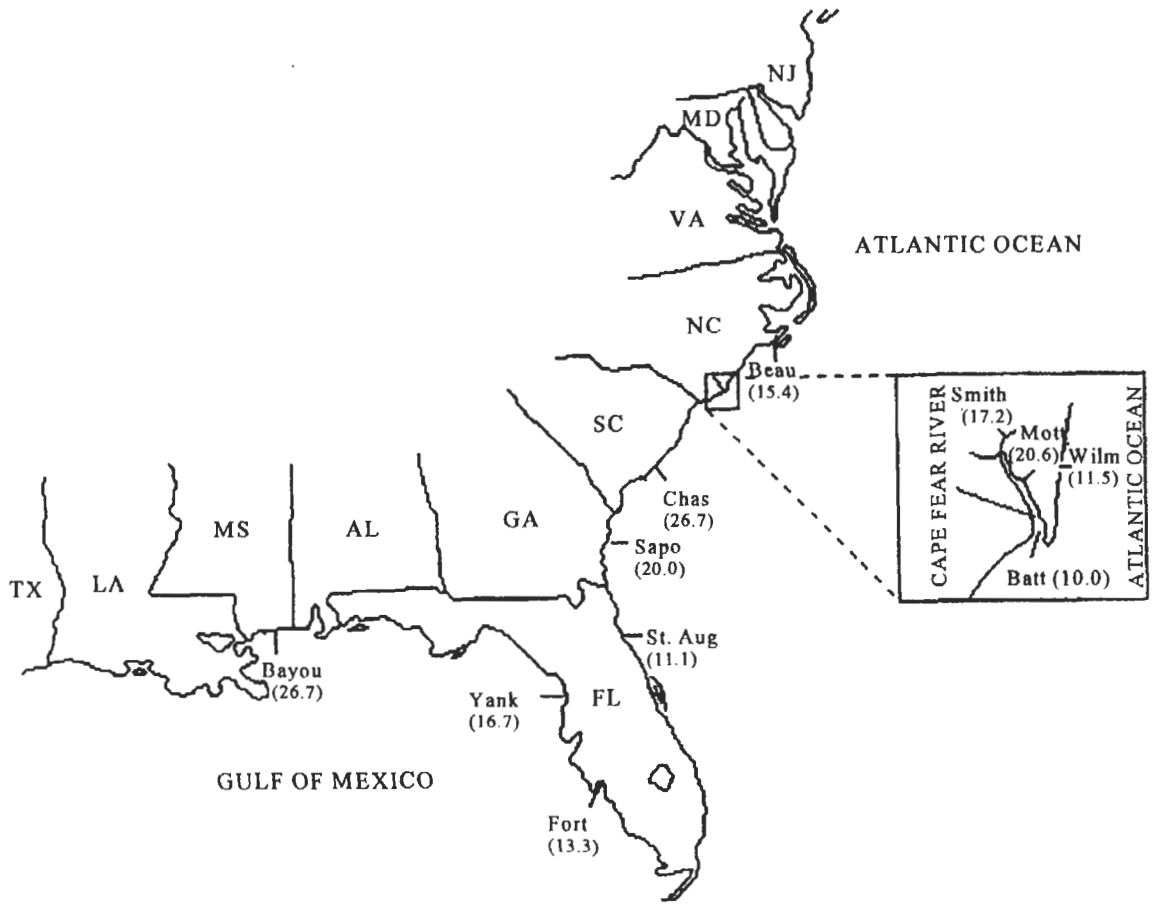

Figure 1. Location of collection sites of *Polymesoda caroliniana* along the Gulf and Atlantic coasts of the United States. Inset shows the location of four collection sites in the Cape Fear Estuary, NC. Sites are identified as follows: Beau = Beaufort, NC; Wilm = Wilmington Intracoastal Waterway, NC; Smith = Smith Creek, NC; Mott = Mott Creek, NC; Batt = Battery Island, NC; Chas = Charleston County, SC; Sapo = Sapelo Island, GA; St. Aug = St. Augustine, FL; Fort = Fort Myers, FL; Yank = Yankeetown, FL; Bayou = Ocean Springs, MS. Numbers in parentheses represent the geographic distribution of the percentage of heterozygotes at the *Gdh* locus.

Carolina to Mississippi (Figure 1). Specific site descriptions are as follows: Beaufort, NC (34.73 N): Intertidal marsh dominated by *S. alterniflora* and *J. roemerianus* along Bell Creek, a tributary of the Newport River, on the west side of County Road 1161 approximately 15 km north of Beaufort, NC, in Carteret County; Wilmington, NC (34.17 N): Intertidal marsh dominated by *S. alterniflora* and *J. roemerianus* along the Intracoastal Waterway, approximately 10 km south of Wilmington, NC, in New Hanover County. This site is along County Road 1492 approximately 4 km south of Whiskey Creek; Smith Creek, NC (34.25 N): Intertidal marsh dominated by *Spartina cynosuroides* along Smith Creek, a tributary of the Northeast Cape Fear River in New Hanover County, NC. Mott Creek, NC (34.15 N): Intertidal marsh dominated by *Scirpus olneyi* and *J. roemerianus* along Mott Creek on the west side of North Carolina Highway 1100, 14 km south of Wilmington, NC, in New Hanover County; Battery Island, NC (33.92 N): Intertidal marsh dominated by *S. alterniflora* on Battery Island, NC, in Brunswick County. The population was located approximately 100 m from the Cape Fear River on the south side of the island; Charleston, SC (32.85 N): Intertidal marsh dominated by *J. roemerianus* located where a tidal creek crosses U. S. Highway 17, 25 km north of the intersection of U. S. Highway 17 and Highway 41, north of Charleston, SC, in Charleston County; Sapelo Island, GA (31.48 N): Intertidal marsh dominated by *S. alterniflora* and *J. roemerianus* at the upper end of the Duplin River drainage basin on the west side of Sapelo Island, in Macintosh County; St. Augustine, FL (29.92 N): Subtidal population in Pellicer Creek with an average depth of 10 cm at low tide. The collecting site was under a bridge on U. S. Highway 1 that separates St. Johns and Flagler County; Fort Myers, FL (26.45 N): Intertidal mangrove island dominated by *R. mangle* located approximately 10 m southeast of a bridge where State

Highway 867 crosses Whiskey Creek, in Lee County; Yankeetown, FL (29.03 N): Intertidal, well-flooded marsh dominated by *J. roemerianus* approximately 6 km west of Yankeetown, FL, in Levy County, adjacent to a picnic area; Ocean Springs, MS (30.41 N): Intertidal marsh dominated by *J. roemerianus* located in Bellfontaine marsh south of Davis Bayou which is east of Ocean Springs, MS, in Jackson County.

Sample Processing

Horizontal starch gel electrophoresis was employed to detect genetic variation at eight enzyme loci and five general protein loci in *P. caroliniana.* At least 30 adult clams were assayed from each site with the exception of the Beaufort, NC, site (n=29; Table 1). Foot tissue was used in all assays because in *P. caroliniana* it is predominantly muscle and contains little if any visceral mass (Grater, personal observation). The whole foot was excised, weighed on a Mettler PC 2200 electronic balance (±0.05 g), ground 35 strokes in 0.5 mL of cold Tris/HCl, pH 8.0, in a Deltaware No. 96014 one mL tissue grinder, and centrifuged at 10,500 g for 20 min at 4°C in a Beckman Model J2-21 M/E centrifuge. Tracking dye (0.05 mL 0.2% bromophenol blue) was added to the supernatant fluid. Samples were then applied to two 5 mm x 5 mm Whatman 3MM chromatography paper wicks, blotted on a sheet of paper toweling to remove excess liquid and inserted into a slit cut in the gel. Each gel held 16 samples (15 data samples and a control). The control was a sample of a known migration rate from a previous gel.

Electrophoresis buffers and running and staining procedures were based on the methods of Selander et al. (1971), Harris and Hopkinson (1976) and Schaal and Anderson (1974). Based on preliminary trials, histochemical stains specific for seven enzymes — sorbital dehydrogenase (SORDH; EC No. 1.1.1.14), malate dehydrogenase (MDH; EC No. 1.1.1.37), malicenzyme (ME; EC No. 1.1.1.40), glucose dehydrogenase (GDH; EC No. 1.1.1.47), glucose-6-phosphate dehydrogenase (GD; EC No.1.1.1.49), glutamate-oxaloacetate transaminase (GOT; EC No. 2.6.1.1) and esterase (EST; EC No. 3.1.1.1) — and one general protein stain (Ptn) were employed in this study.

Statistical Analyses

Multiple loci encoding the same enzyme were designated by consecutive numbers, with "1" denoting the slowest migrating enzyme. Alleles within each locus were scored by designating the most common allele as 100. Other alleles were numbered according to their relative anodal distance from the most common allele. *Got* electromorphs resolved into distinct zones but not always into distinct genotypes and thus were lumped into the following classes: 1) homozygotes of the most common allele, 2) heterozygotes with the most common allele, and 3) all other genotypes. The five proteins visualized with general protein stain were presumably nonenzymatic muscle contractile proteins and were distinguished as individual loci based on the irregularity of the spacing between electromorphs, the homogeneity of staining intensity of electromorphs, and the reproducibility of these results.

The percentage of individual allele types at each locus was calculated following Hartl (1988). A locus was considered monomorphic if the frequency of the least common allele was less than 1% (Hartl 1988). The average percentage of loci that were heterozygous in an individual was determined for each population following Hartl (1988).

Independence of allele associations was tested with two-way and multiway contingency table analyses using the G-statistic (Sokal and Rohlf 1995). All possible pairwise combinations of loci within each population were examined. The G-test, analogous to the Chi-square test (Sokal and Rohlf 1995), should not be used when classes contain fewer than five observations (Hartl 1988). When numbers in an allele class were less than five, classes were combined as follows. In diallelic enzyme systems, heterozygotes were lumped with the smaller homozygous class. In multiple allele systems, the following three classes were created: 1) homozygotes of the most common allele, 2) heterozygotes with the most common allele, and 3) all other genotypes.

Two tests were used to determine whether proportions of genotypes conformed to Hardy-Weinberg expectations. Haldane's (1954) exact test for randomness of mating was used for diallelic systems and for multiple allele systems for populations in which only two alleles were observed. Where multiple allele systems were found, the Chi-square test of Hardy-Weinberg expectations was used (Hartl 1988). However, in multiple allele systems, numbers in expected classes were frequently less than five and combining of classes was necessary.

A G-test was employed to test heterogeneity of allele distributions for each locus in all populations (Sokal and Rohlf 1995). Where heterogeneity was observed, the source of the heterogeneity was determined by calculating a G-statistic for each population. G-tests and Hardy-Weinberg analyses were corrected for a-errors associated with repeated testing using the Bonferroni correction (Sokal and Rohlf 1995).

Three levels of population structure were estimated with F-statistics: individual within subpopulation (F_{IS},

TABLE 1

Number of animals scored at each locus. Sites are identified as in the legend of Figure 1.

Locus and Electromorph	Populations										
	Beau, NC	Wilm, NC	Smith, NC	Mott, NC	Batt, NC	Chas, SC	Sapo, GA	St. Aug, FL	Fort, FL	Yank, FL	Bayou, MS
Sordh	8	--[a]	30	37	--	--	--	--	--	30	24
Mdh	29	30	30	91	52	30	38	51	45	30	30
Me	24	30	30	66	52	30	35	29	45	30	30
Gdh	26	30	29	34	52	30	15	36	45	30	30
Gd	17	28	30	36	41	29	--	35	37	30	29
Got	21	--	--	--	52	30	38	36	26	22	--
Est-1	28	28	28	38	17	30	14	35	30	29	29
Est-2	24	29	--	36	7	28	9	31	28	28	26
Ptn-1	--	--	30	30	--	30	15	--	15	30	15
Ptn-2	--	--	30	30	--	30	15	--	15	30	15
Ptn-3	29	--	--	37	37	30	19	36	45	15	15
Ptn-4	29	--	--	37	37	30	19	36	45	15	15
Ptn-5	--	15	--	10	7	30	--	20	13	15	30

[a]Dashed line indicates no data collected

positive values indicate heterozygote deficiencies), subpopulation within region (F_{IT}), and region within total area sampled (F_{ST}). *F*-statistics were conducted using Genetic Data Analysis software (Lewis and Zoykin 1997).

Genetic differentiation between populations was estimated with Nei's unbiased estimates of genetic identity and genetic distance (Nei 1987). Nei's genetic identity estimates the proportion of alleles identical in two populations, and Nei's genetic distance estimates the mean number of net codon substitutions between two populations, assuming that individual mutation rates are similar and individual substitution events are independent (Nei 1987). These estimates were conducted using Gendis software (Department of Genetics, University of Georgia; Masatoshi Nei, Institute of Molecular and Evolutionary Genetics, University of Pennsylvania).

A matrix of Nei's genetic distance values was used to construct a tree of relatedness. The method employed was the average distance method or unweighted-pair group method with arithmetic mean (UPGMA), which assumes that the rate of gene substitution is constant (Nei 1987). The BMDP software used was supplied by the University of Georgia.

RESULTS AND DISCUSSION

At several loci, allele frequencies were dissimilar between the two populations from the Gulf coast of Florida (Fort Myers and Yankeetown) and all other populations (Table 2). For both Fort Myers and Yankeetown populations, at the *Gdh* locus, the frequency of the 100 allele was highest and the frequencies of the 106 and 113 alleles lowest. At the *Me* locus, Fort Myers and Yankeetown alleles were fixed for the same allele; however, the Smith Creek, NC, population was also fixed for this allele. For the 100 allele at the *Gd*, *Got*, and *Est-2* loci, frequencies at Fort Myers were highest.

Davis Bayou, MS, allele frequencies were more similar to those populations from the East coast than to those of other populations from the Gulf coast at all loci except *Mdh*. Alleles unique to the Gulf coast were observed at this locus (Table 2). Allele frequencies did not consistently increase or decrease at four sites along the salinity gradient in the Cape Fear Estuary, or along the latitudinal gradient.

There were sufficient data in allele classes to conduct 13 tests of 65 possible for independence of allele association. No tests were significant at $p<0.05$, indicating lack of linkage or other co-selection for these locus pairs.

Adjusted estimates of percent heterozygosity and percent of polymorphic loci are similar to those of the bivalves *Rangia cuneata* and *R. flexuosa* (Foltz et al. 1995). Heterozygosity ranged between 8.1% at Fort Myers and 28% at Wilmington, NC (Table 3), where data were not obtained for four of the five nonenzymatic loci. Since non-enzymatic loci were monomorphic (Table 2), this heterozygosity estimate would probably be lower if monomorphic data were obtained. The other nine heterozygosity values were all between 14.0 and 21.6 % (Table 3).

The percentage of polymorphic loci ranged between 37.5% at Smith Creek and 71.4% at Wilmington. The other nine estimates were between 50.0 and 60.0% (Table 3). Estimates were generally higher at sites in which data were not obtained for several nonenzymatic loci (Table 2; Table 3). The estimate for Smith Creek could be too low, for gels were unscorable at two loci polymorphic at other sites, and allele frequencies were fixed at three other enzymatic loci, more than at any other site (Table 2).

Populations of *P. caroliniana* were in Hardy-Weinberg equilibrium at greater than 95% of all loci scored other than *Gdh*. Only the populations from Fort Myers and Sapelo Island met Hardy-Weinberg expectations at this locus (Table 3). When combining allele classes to test for Hardy-Weinberg equilibrium at the *Gdh* locus, it would have been possible to obtain significance and nonsignificance of the Chi-square depending on how classes were combined. However, overall low numbers of heterozygotes at the *Gdh* locus indicate these populations actually do not meet Hardy-Weinberg expectations (Figure 1; Table 4), which suggests selection is operating against them, for if inbreeding were occurring, other loci would have failed to conform to Hardy-Weinberg expectations.

For the Fort Myers population, the frequency of the 100 allele was so high (0.91) that expected numbers of heterozygotes containing alleles other than 100 were low, often near zero. This is consistent with the low observed numbers of heterozygotes, and the resulting Chi-square value was not significant (Table 3). The Sapelo Island population conformed to Hardy-Weinberg expectations only after the Bonferroni correction was employed.

In this study, populations of *P. caroliniana* were sampled along a salinity gradient in the Cape Fear Estuary, NC, and along latitudinal gradient from North Carolina to Mississippi, but allele frequencies showed no clinal pattern with either salinity or latitude (Table 2). This, coupled with results of Hardy-Weinberg analysis, suggests there is gene flow among populations within and between estuaries along the Atlantic coast, i.e., *P. caroliniana* are effective at dispersal (Table 3). Also, electrophoretic patterns observed in this study are similar to those of other marine invertebrates with planktonic larvae, i.e., low levels of variation exist among populations (Crisp 1978, Liu et al. 1991, Hoskin 1997). *F*-statistics do indicate there is some population

TABLE 2

Allele frequencies observed in 11 populations of *Polymesoda caroliniana*. Sites are identified as in the legend of Figure 1.

Locus		Beau, NC	Wilm, NC	Smith, NC	Mott, NC	Batt, NC
Sordh	106		-----[a]		0.030	-----
	100	1.0	-----	1.0	0.97	-----
Mdh	105					
	100	1.0	1.0	1.0	1.0	1.0
	94					
	89					
Me	100	0.69	0.92	1.0	0.91	0.79
	82	0.31	0.080		0.090	0.21
Gdh	119	0.080	0.050		0.060	0.10
	113	0.23	0.33	0.39	0.16	0.29
	106	0.42	0.22	0.14	0.26	0.21
	100	0.23	0.40	0.45	0.52	0.38
	89	0.040		0.020		0.020
	82					
Gd	100	0.53	0.29	0.68	0.60	0.70
	87	0.47	0.71	0.32	0.40	0.30
Got	100	0.64	-----	-----	-----	0.78
	Variant	0.36	-----	-----	-----	0.22
Est-1	100	0.45	0.48	0.61	0.54	0.65
	95	0.55	0.52	0.39	0.46	0.35
Est-2	100	0.58	0.47	-----	0.46	0.64
	96	0.42	0.53	-----	0.54	0.36
Ptn-1		-----	-----	1.0	1.0	----
Ptn-2		-----	-----	1.0	1.0	1.0
Ptn-3		1.0	-----	-----	1.0	1.0
Ptn-4		1.0	-----	-----	1.0	1.0
Ptn-5		-----	1.0	-----	1.0	----

[a]Dashed line indicates no data collected

TABLE 2 (Continued)

Locus		Chas, SC	Sapo, GA	St. Aug, FL	Fort, FL	Yank, FL	Bayou, MS
Sordh	106	-----	-----	-----	-----	0.030	
	100	-----	-----	-----	-----	0.97	1.0
Mdh	105					0.030	
	100	1.0	1.0	1.0	0.99	0.92	0.98
	94					0.030	
	89				0.010	0.020	0.020
Me	100	0.87	0.93	0.91	1.0	1.0	0.98
	82	0.13	0.070	0.090			0.020
Gdh	119	0.070	0.10	0.070		0.030	0.030
	113	0.30	0.20	0.31	0.050	0.070	0.17
	106	0.22	0.23	0.22	0.010	0.13	0.25
	100	0.30	0.47	0.35	0.91	0.75	0.43
	89	0.080		0.050	0.030	0.020	0.050
	82	0.030					0.070
Gd	100	0.50	-----	0.66	0.80	0.40	0.64
	87	0.50	-----	0.34	0.20	0.60	0.36
Got	100	0.65	0.70	0.56	0.88	0.77	-----
	Variant	0.35	0.30	0.44	0.12	0.23	-----
Est-1	100	0.42	0.71	0.51	0.73	0.62	0.55
	95	0.58	0.29	0.49	0.27	0.38	0.45
Est-2	100	0.45	0.67	0.44	0.89	0.70	0.64
	96	0.55	0.33	0.56	0.11	0.30	0.36
Ptn-1		1.0	1.0	-----	1.0	1.0	1.0
Ptn-2		1.0	1.0	-----	1.0	1.0	1.0
Ptn-3		1.0	1.0	1.0	1.0	1.0	1.0
Ptn-4		1.0	1.0	1.0	1.0	1.0	1.0
Ptn-5		1.0	-----	1.0	1.0	1.0	1.0

TABLE 3

Chi-square values for Hardy-Weinberg analysis, percent heterozygosity and percent loci polymorphic for 11 populations of *Polymesoda caroliniana*. Sites are identified as in the legend of Figure 1.

Site	Chi-square values for Hardy-Weinberg Equilibrium								Heterozygosity	Loci Polymorphic
	Locus									
	Sordh	*Mdh*	*Me*	*Gdh*	*Gd*	*Got*	*Est-1*	*Est-2*	(%)	(%)
Beau, NC	0.00	0.00	4.47	23.5*	5.14	4.88	3.72	0.522	21.6	60.0
Wilm, NC	-----[a]	0.00	0.192	33.5*	4.07	-----	0.215	2.55	28.0	71.4
Smith, NC	0.00	0.00	0.00	15.4*	2.55	-----	0.0250	-----	15.5	37.5
Mott, NC	0.00196	0.00	0.596	14.9*	0.245	-----	6.96	1.09	14.0	50.0
Batt, NC	-----	0.00	3.51	19.7*	0.0475	1.56	4.43	0.137	19.0	60.0
Chas, SC	-----	0.00	0.602	33.0*	0.417	7.55	2.04	5.03	18.8	50.0
Sapo, GA	-----	0.00	0.172	8.14	-----	0.231	0.761	0.300	14.8	50.0
St. Aug, FL	-----	0.00	0.200	38.8*	2.23	1.82	0.558	0.816	18.3	60.0
Fort, FL	-----	0.00	0.00	0.665	6.91	2.04	3.38	0.342	8.11	50.0
Yank, FL	0.0170	21.2*	0.00	16.4*	0.998	1.36	0.00170	0.221	14.4	53.8
Bayou, MS	0.00	0.0170	0.00	16.8*	0.0640	-----	1.24	1.29	16.6	50.0

*$p<0.05$

[a]Dashed line indicates no data collected

TABLE 4

***F*-statistics of 11 populations of *Polymesoda caroliniana*. The estimates of three levels of population structure are as follows: F_{IS} = individual within subpopulation, F_{IT} = subpopulation within region, and F_{ST} = region within total area sampled.**

Locus	F_{IS}	F_{IT}	F_{ST}
Sordh	-0.150	0.905	0.907
Mdh	0.428	0.443	0.259
Me	-0.135	-0.033	0.090
Gdh	0.731	0.752	0.077
Gd	0.050	0.118	0.072
Est-1	0.153	0.158	0.006
Est-2	-0.050	0.025	0.071
Overall	0.235	0.323	0.115

subdivision within regions, but differentiation over the entire study area is moderate (Table 4). Our results confirm Olsen's (1976) observation that *P. caroliniana* larvae are planktonic and lend support to Hackney's (1983) hypothesis that *P.caroliniana* larvae are capable of long-term residence as meroplankton.

Electrophoretic patterns between *P. caroliniana* populations from the Atlantic and Gulf coasts are unique when compared with those of many other studies. Numerous investigators have described distinct genetic differences between Atlantic and Gulf populations of many species using various genetic markers (Reeb and Avise 1990, Avise 1992, Karl and Avise 1992, Sarver and Foltz 1992, Felder and Staton 1994, Foltz et al. 1995). In this study, heterogeneity analyses and dendrograms, based on allele frequencies, indicate that populations assayed from the west coast of Florida (Fort Myers and Yankeetown) are genetically distinct and physically isolated from other populations assayed, and that a population from Mississippi is genetically more similar to Atlantic coast populations than to west coast Florida populations (Table 5a and b; Figure 2a and b).

Heterogeneity analyses revealed significant heterogeneity in population allele frequencies at each of five loci: *Me*, *Gdh*, *Gd*, *Got* and *Est-2* (Table 5a). Much of this heterogeneity was due to populations from the west coast of Florida, with clams from Fort Myers significantly heterogeneous at all five loci and clams from Yankeetown at three (Table 5b). Two loci were heterogeneous in clams from Beaufort and Smith Creek, while clams from Wilmington, Battery Island and Davis Bayou were heterogeneous at one locus (Table 5b).

In this study, data were obtained for all 11 populations at only four enzymatic loci (Table 2); therefore, a dendrogram was produced with data from only these four loci (Figure 2a). In this dendrogram, the maximum genetic distance (D) separating populations other than Beaufort, Fort Myers and Yankeetown was 0.02. Beaufort was separated from this group by D=0.05. Fort Myers and Yankeetown were separated from each other by D=0.01, and from all other populations by D=0.06. These three populations represent the northern and southern extremes of the study area.

Another dendrogram was produced, using six loci (Figure 2b), by excluding two populations in which there were missing data, Smith Creek and Sapelo Island (Table 2). In this dendrogram, the maximum D separating populations other than Fort Myers and Yankeetown was 0.04. Fort Myers and Yankeetown were separated from each other by D=0.05, and these two populations were separated from all others by D=0.08.

Numerous models have been employed to explain genetic differences and similarities between Gulf and Atlantic populations (Bert 1986, Reeb and Avise 1990, Avise 1992, Karl and Avise 1992, Felder and Staton 1994) and each could be invoked in some form here. Most notably, in an analysis of restriction fragment length polymorphisms in single-copy nuclear DNA of the American oyster, *Crassostrea virginica*, Karl and Avise (1992) found pronounced discontinuities between Gulf and Atlantic populations. These findings were similar to those of a mitochondrial DNA (mtDNA) survey of *C. virginica* (Reeb and Avise 1990), but were contradictory to those of an allozyme survey of *C. virginica* in which little or no population subdivision between Gulf and Atlantic populations was observed (Buroker 1983). Also, mtDNA surveys of other coastal taxa, including horseshoe crabs, toadfish, black sea bass, diamondback terrapins, and seaside sparrows (Avise 1992) reveal pronounced discontinuities between Gulf and Atlantic populations. Karl and Avise (1992), Avise (1992), and Reeb and Avise (1990) suggest this subdivision occurs as a result of vicariant historical processes, and Karl and Avise (1992) suggest the homogeniety of allozyme polymorphisms observed in *C. virginica* may be the result of balancing selection, which can counter the influence of genetic drift.

In the current study, only three Gulf coast sites were sampled and dendrograms are based on data from only four to six loci; therefore, genetic relationships observed in the dendograms must be considered preliminary (Nei 1987). One possible explanation may be that the existence of the Suwanee Strait or Gulf Trough, which isolated peninsular Florida from the continental US, may have facilitated gene

TABLE 5a

Heterogencity G-test values for 11 populations of *Polymesoda caroliniana* pooled.

	Locus							
	Sordh	*Mdh*	*Me*	*Gdh*	*Gd*	*Got*	*Est-1*	*Est-2*
df	4	30	10	50	9	6	10	9
$G_{Heterogeneity}$	5.34	31.10	79.87*	203.4*	54.77*	22.23*	17.55	35.68*
G_{Pooled}	0.001800	0.4044	-0.04260	0.02240	-0.01000	-0.02000	-0.04600	-0.02000
G_{Total}	5.342	31.50	79.83*	207.6*	54.76*	22.21*	17.51	35.66*

*p<0.05

TABLE 5b

Heterogeneity G-test values for individual populations of *Polymesoda caroliniana*. Sites are identified as in the legend of Figure 1.

Site	Locus				
	Me	*Gdh*	*Gd*	*Got*	*Est-2*
Beau, NC	18.57*	19.97*	0.5592	0.9136	0.002000
Wilm, NC	0.03900	8.367	21.61*	-----[a]	3.489
Smith, NC	11.45*	15.02*	2.090	-----	-----
Mott, NC	00.007200	7.903	0.004200	-----	4.853
Batt, NC	13.77*	9.506	3.674	2.442	0.1790
Chas, SC	1.149	13.12*	2.036	1.048	-8.137
Sapo, GA	0.3430	3.583	-----	0.06360	0.4776
St. Aug, FL	0.01140	8.174	1.218	7.819	5.785
Fort, FL	17.17*	85.10*	14.03*	9.071*	25.69*
Yank, FL	11.45*	19.94*	9.034*	0.8512	2.844
Bayou, MS	5.879	12.86*	0.4918	-----	0.4814
G_{Total}	79.83*	203.5*	54.75*	22.21*	35.66*

*p<0.05

[a]Dashed line indicates no data collected

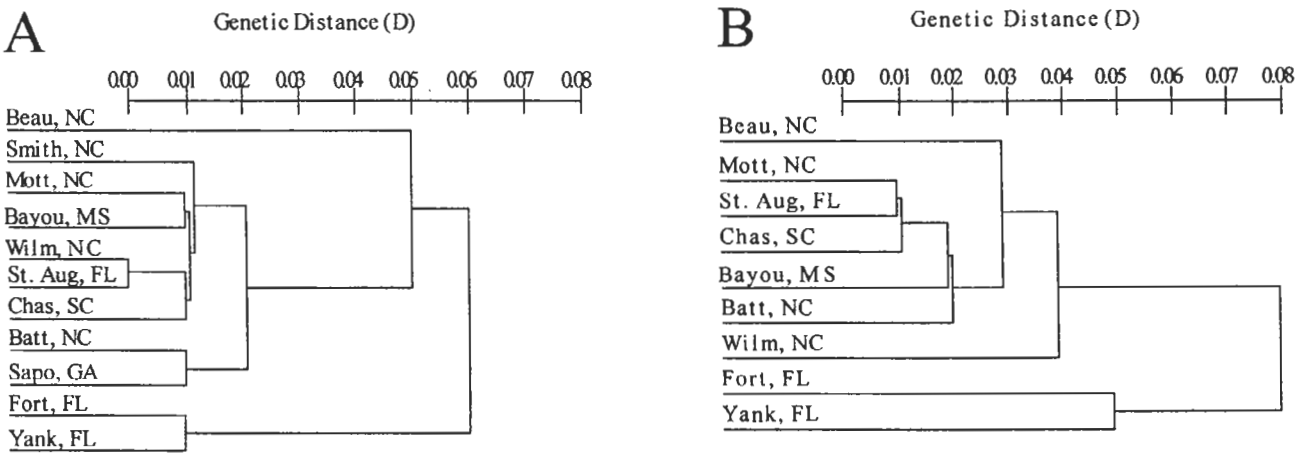

Figure 2. (A) UPGMA dendrogram based on electromorph frequencies at four loci (*Mdh*, *Me*, *Gdh*, and *Est-1*) in each of 11 populations of *Polymesoda caroliniana*. (B) UPGMA dendrogram based on electromorph frequencies at six loci (*Mdh*, *Me*, *Gdh*, *Gd*, *Est-1*, and *Est-2*) in each of nine populations of *Polymesoda caroliniana*. Sites are identified as in the legend of Figure 1.

flow between the Mississippi population and Atlantic coast populations, while isolating west coast Florida populations. The existence of the Suwanee Strait is controversial, and Webb (1990) notes that biological arguments for the existence of the Suwanee Strait ". . . based on various degrees of endism in the biota of the central peninsula, would be just as well satisfied by the existence of habitat islands as by the existence of a hypothetical seaway to produce real islands."

A second explanation may be with the onset of glaciation, populations of *P. caroliniana* from the isolated southern portion of the range may have retreated south to the Yucatan or the larger Caribbean island subtropical habitat where some genetic divergence may have occurred. With glacial retreat and the gradual northern movement of ecosystems these populations may have returned to Florida along with subtropical habitat. The presence of alleles unique to Gulf coast populations suggests there once was, and/or currently is, gene flow between them.

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