Range-wide population structure and history of the northern quahog (*Merceneria merceneria*) inferred from mitochondrial DNA sequence data

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The northern quahog (*Mercenaria mercenaria*) is a commercially important bivalve distributed in the NW Atlantic from Florida to Nova Scotia. We report on population genetic analyses, based on 528 bp of mitochondrial COI gene, for 10 locations across the range of *M. mercenaria* ($n = 297$). Our analyses revealed no evidence of cryptic evolutionary lineages, but modest population structure ($\varphi_{ST} = 0.0213$; $p = 0.0019$) with a significant partition at Cape Hatteras and a weakly supported partition at Cape Cod. Samples from the west coast of Florida (Gulf of Mexico) were not significantly different from most Atlantic populations, despite a 600 km gap in distribution along South Florida, and a well-documented biogeographic break at Cape Canaveral. These findings support the thesis that the Gulf of Mexico population is the product of a recent introduction. Samples north of Hatteras decrease in diversity with increasing latitude, probably indicating post-glacial range extension, a conclusion supported by a highly significant Fu's *F*-statistic (-99.14; $p < 0.001$) indicating population expansion. *Mercenaria mercenaria* stocks in the Atlantic include a single evolutionary unit divided into at least three closely related populations, though this does not preclude regional adaptive differences between northern, central, and southern populations.

Keywords: biogeography, clam, conservation genetics, cytochrome oxidase I, mollusc, Northwest Atlantic, phylogeography.

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Introduction

A major area of research for exploited marine species is in resolving management units with population genetic tools. Genetic studies can identify discreet stocks and patterns of historical and contemporary gene flow, even in species displaying high dispersal capabilities (Waples, 1998). Genetic approaches also provide insight into population demographic processes and the role of selection. Disentangling these processes in marine species is particularly relevant in light of the potential for interactive effects of exploitation and local and global climatic change on species with high variance in reproductive success (O'Brien *et al.*, 2000).

Repeated episodes of global cooling during the Pleistocene resulted in water temperature fluctuations, alterations in upwelling patterns, and sea level changes (Hays *et al.*, 1976; Bond *et al.*, 1997) that had profound impacts on coastal marine species. Genetic data can often illuminate the impact of past climatic events and current harvest and aquaculture practices.

The northern quahog or hard clam (*Mercenaria mercenaria*) is an important fishery and aquaculture species (Mackenzie *et al.*, 2001) that ranges from the Gulf of St Lawrence in Canada to the Indian River Lagoon in east Florida, USA, with disjunct occurrences in the Gulf of Mexico (Woodburn, 1961; Harte, 2001; Arnold *et al.*, 2004). North of Massachusetts, USA (approximately 41°N), the distribution of *M. mercenaria* is discontinuous, and there is no commercial fishery between Cape Cod and the Gulf of St Lawrence (Harte, 2001). However, *M. mercenaria* is abundant in the Gulf of St Lawrence (~45°N).

Mercenaria mercenaria is a protandrous hermaphrodite that does not normally reproduce as a female (at least in the northern portion of its range) until it is at least 2–3 years old; animals may live for decades and older females contribute significantly to the population reproductive output (Eversole, 2001). Fertilization is external and followed by a planktonic larval phase of 6–8 d (Carriker, 1951; Eversole, 2001). High potential gene flow has been associated repeatedly with species having planktonic larval stages, and such species typically show little genetic structure at regional or larger scales (Hellberg *et al.*, 2002). Exceptions to this pattern may arise, however, as a result of environmental dynamics, historical events, biological mechanisms, or anthropogenic factors (Barber *et al.*, 2000; Nelson *et al.*, 2000). For example, the discontinuous distribution of *M. mercenaria* north of Cape Cod is mirrored by that of the eastern oyster (*Crassostrea virginica*; Abbott, 1986). Cape Hatteras, North Carolina, and Cape Canaveral, Florida, are also documented biogeographic breaks in the distribution of coastal benthic invertebrates (Saunders *et al.*, 1986; Reeb and Avise, 1990; Calder,

1992; Sarver *et al.*, 1992; Hare and Avise, 1998; Engle and Summers, 1999).

Despite its commercial importance, no studies have examined the spatial scale and patterns of genetic connectivity, so here we examine mtDNA sequence variation across the distribution of *M. mercenaria*. Our primary goal is to resolve the species' population structure and biogeographic history. However, we also assess a possible introduction into the Gulf of Mexico, and test for cryptic evolutionary partitions that are commonly revealed in genetic surveys of molluscs (King *et al.*, 1999; Lee and Ó Foighil, 2004; Meyer *et al.*, 2005).

Material and methods

Sampling

We conducted a range-wide survey of 297 *M. mercenaria* from 10 locations (Figure 1), including two samples from the Cedar Key area of west Florida (Table 1). Those two samples are from an area of intensive commercial culture of the species and may represent aquaculture escapes (Adams and Sturmer, 2004). Samples were obtained from commercial fishery sources (North Carolina through Prince Edward Island), or collected by the authors or volunteers (Georgia and Florida locations). Sites were selected to bracket biogeographic boundaries at Cape Canaveral (Florida), Cape Hatteras (North Carolina), and Cape Cod (Massachusetts).

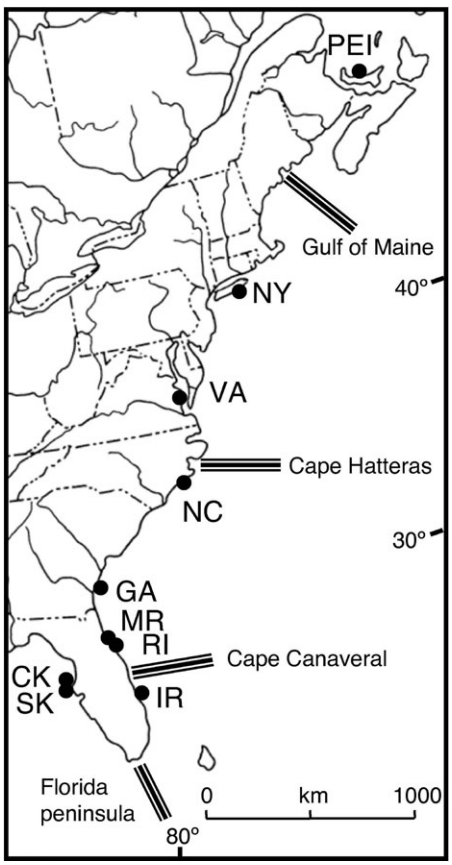

Figure 1. Distribution of the 10 sampled locations for *M. merceneria*. Abbreviations follow those in Table 1. Triple (thick) lines indicate putative phylogeographic breaks that have either been associated with other coastal marine invertebrate species or that are presumed to exist given the known distributions.

Shells and tissue vials were marked with specimen codes and archived at the University of Florida, Department of Fisheries and Aquatic Sciences.

Mitochondrial DNA surveys

Total DNA was extracted from 25 μg of ethanol-preserved adductor tissue using QIAGEN® DNeasy® tissue kits (Qiagen Inc., Valencia, CA, USA). A fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was initially amplified using metazoan polymerase chain reaction (PCR) primers developed by Folmer *et al.* (1994). From this fragment, *M. mercenaria*-specific PCR primers were developed (5′ TTTTCTATTTGG GCAGGTCT 3′ and 5′ CCTAACCCTACAGGATCAAAA 3′), yielding a fragment of 542 bp after alignment.

PCR amplifications were conducted in 25 μl reactions containing 1× concentration of PCR buffer (Promega), 2.5 mM $MgCl_2$, 10 mM deoxyribonucleotide triphosphate, 0.5 U Promega *Taq*, and approximately 100 μg of template DNA. PCR reactions consisted of an initial denaturation cycle at 94°C (1 min) followed by 35 cycles consisting of 94°C (1 min), 48°C (1 min), 72°C (1 min), and a final extension at 72°C for 6 min. A negative control was included in each reaction. Unincorporated nucleotides and primers were removed using ExoSAP-IT®. Sequencing reactions used Big Dye terminator sequencing chemistry (Applied Biosystems, Perkin Elmer, Foster City, CA, USA). Sequences were cleaned with Sephadex® (Sigma-Aldrich) and electrophoresed on an Applied Biosystems 3100 Genetic Analyzer. DNA strands were verified, aligned, and joined into contiguous sequences using SEQUENCHER (ver. 4.2, Gene Codes Corp., Ann Arbor, MI, USA).

mtDNA analysis

Relationships among haplotypes were estimated using statistical parsimony (Templeton and Sing, 1993) implemented in TCS 1.18 (Clement *et al.*, 2000). The method reconstructs relationships among intraspecific haplotypes more accurately than traditional tree-based methods, particularly when variable characters are few and ancestral haplotypes are common (Watterson and Guess, 1977; Crandall, 1994). We tested for deviations from neutral evolution using the *F*-statistic of Fu (1996) calculated in DNasP ver. 4.0 (Rozas *et al.*, 2003). When significant, this test indicates population expansion for neutral or near-neutral evolving markers like mtDNA and is more powerful than other available tests (Ramos-Onsins and Rozas, 2002). Indices of genetic diversity were quantified using Arlequin ver. 3.1 (Excoffier *et al.*, 2005). For each population sample, we calculated haplotype diversity (h) following Nei (1987), the probability that two randomly selected haplotypes in a sample are different (without consideration of their evolutionary relationship), and its standard deviation. We also measured nucleotide diversity (π), the mean sequence divergence between individuals (Tajima, 1993). These estimates were used to calculate the corrected between-sample π that accounts for diversity within samples $[\pi_{net} = \pi_{XY} - 0.5[\pi_X + \pi_Y]]$. We also applied the correction $[p_{net} = p_{XY} - 0.5(p_X + p_Y)]$ to Kimura two-parameter distances (Kimura, 1980) among all pairwise samples calculated in Mega 3.0 (Kumar *et al.*, 2004). Corresponding genetic distances among samples were visualized in a distance tree constructed using the Fitch–Margoliash criterion as implemented in the Fitch subroutine of Phylip version 3.5 (Felsenstein, 1993).

Table 1. *Mercenaria merceneria* sample locations, sample sizes (*n*), haplotype diversity (*h*) (s.d.=standard deviation), number of segregating sites (*S*), and haplotype identifier (frequency).

Locality	Code	Latitude and longitude	*n*	*h* (s.d.)	*S*	Haplotypes
Charlottetown, PEI[1]	PEI	46°2.90′N 63°8.35′W	33	0.7595 (0.053)	7	1(14), 12(6), 13(3), 75(3), 76(6), 77(1)
Patchogue Bay, NY[2]	NY	40°40.61′N 73°9.91′W	32	0.7863 (0.065)	14	1(14), 12(1), 13(2), 39(3), 40(1) 41(2), 42(5), 43(1), 44(1), 45(1), 46(1)
Mobjack Bay, VA[3]	VA	37°16.10′N 76°17.10′W	32	0.8286 (0.064)	26	1(13), 5(1), 9(4), 12(1), 52(1), 55(1), 56(1), 57(2), 58(1), 59(1), 60(1), 61(1), 62(1), 63(1), 64(1), 65(1)
New River, NC[4]	NC	34°32.20′N 77°20.38′W	35	0.9109 (0.035)	25	1(9), 3(6), 5(1), 9(1), 10(2), 20(2), 28(1), 32(1), 33(1) 38 (1), 53(1), 66(1), 67(1), 68(1), 69(1), 70(1), 71(1), 72(1), 73(1), 74(1)
Wassaw Sound, GA[5]	GA	31°56.12′N 80°58.16′W	33	0.8447 (0.048)	21	1(10), 3(9), 5(1), 8(1), 11(1), 29(2) 35(1), 47(1), 48(1), 49(1), 50(1), 51(1), 52(1), 53(1), 54(1)
Matanzas River, FL[6]	MR	29°42.55′N 81°14.05′W	31	0.8129 (0.066)	25	1(13), 3(4), 5(1), 8(1), 9(3), 12(1), 78(1), 79(1), 80(1), 81(1), 82(1), 83(1), 84(1), 85(1)
Rattlesnake Isl., FL[7]	RI	29°40.66′N 81°13.32′W	29	0.8251 (0.059)	18	1(11), 2(1), 3(6), 4(1), 5(2), 6(1) 7(1), 8(1), 9(1), 10(1), 11(1), 12(1), 13(1)
Indian River, FL[8]	IR	28°28.76′N 80°45.14′W	35	0.8504 (0.048)	22	1(12), 2(1), 3(7), 9(1), 11(2), 12(2), 13(1), 30(1), 31(1), 32(1), 33(1), 34(1), 35(1), 36(1), 37(1), 38(1)
Cedar Key, FL[9]	CK	29°6.90′N 83°4.49′W	20	0.9474 (0.034)	16	1(4), 7(1), 9(2), 16(1), 20(1), 21(3), 22(1), 23(1), 24(1), 25(1), 26(1), 27(1), 28(1), 29(1)
Seahorse Key, FL[7]	SK	29°5.69′N 83°3.91′W	17	0.8382 (0.087)	23	1(7), 3(1), 12(1), 14(1), 15(1), 16(1), 17(1), 18(1), 19(1), 29(2)

Sources: [1]J. P.'s Shellfish, [2]Captree Clam Co., [3]J&W Seafoods, [4]Bowman's Seafood, [5]A. Power, [6]J. N. Jonathon, [7]S. Fajans, [8]L. N. Sturmer, [9]P. Baker.

Analysis of molecular variance (AMOVA), also implemented with Arlequin, was performed to test the geographic divisions among sampled populations. AMOVA calculates the proportion of variation among groups (Φ_{CT}), the proportion of variation among populations within groups (Φ_{SC}), and the proportion of variation within populations (Φ_{ST}) (note that we have adopted the Arlequin subscript definitions to define differentiation within and among groups). We explored numerous population groupings based on putative biogeographic barriers. Estimates of pairwise population parameters were calculated with and without Gulf of Mexico samples because of their possible non-native history. We tested the significance of the Φ_{ST} using 10 000 permutations in Arlequin. We also calculated overall and pairwise Φ_{ST} [analogous to Wright's (1965) measure of differentiation, F_{ST}] to determine whether any two samples were genetically differentiated.

Results

Based on 528 bp of COI, we detected 72 variable sites and 85 haplotypes in 297 specimens (Genbank Accession Nos TBA; see Table 1 for haplotype frequencies). The statistical parsimony network reveals a cluster of closely related haplotypes, with the most divergent haplotypes separated by just 16 mutations (Figure 2). Most haplotypes differed by a single base substitution, although a small number of haplotypes found only in the southern samples were diverged by a greater-than-average number of mutations (e.g. Haplotype 3; Figure 2 and Table 1). Numerous unresolved relationships (loops) were present, indicative of the reticulate patterns of evolution common in intraspecific phylogenies.

Haplotype 1 was the most common (107 of 297) overall and in each sampled population, ranging from 20% to 44% frequency. In all, 76 haplotypes were observed in three or fewer animals. Haplotype 3 (and closely associated haplotypes) was notably absent north of Cape Hatteras, but observed in 32 animals from the southern portion of the Atlantic distribution and in one from the Gulf of Mexico. Haplotypes from the ancestral area are expected to retain more ancestral variation with lineage pruning through successive colonization of new locations. The overall haplotype diversity was $h = 0.851$, and the nucleotide diversity was $\pi = 0.0073$, with an average pairwise nucleotide difference of 3.83. Fu's statistic was $F = -99.14$ ($p < 0.0005$), indicating a deviation from neutrality or, assuming near-neutrality (Ohta, 1992), recent population expansion. This is seen in the "star-like" distribution and frequency of haplotypes (Table 1 and Figure 2) indicative of range expansion or population increase (Rogers and Harpending, 1992). Diversity indices h and π varied between populations and both showed a declining trend as a

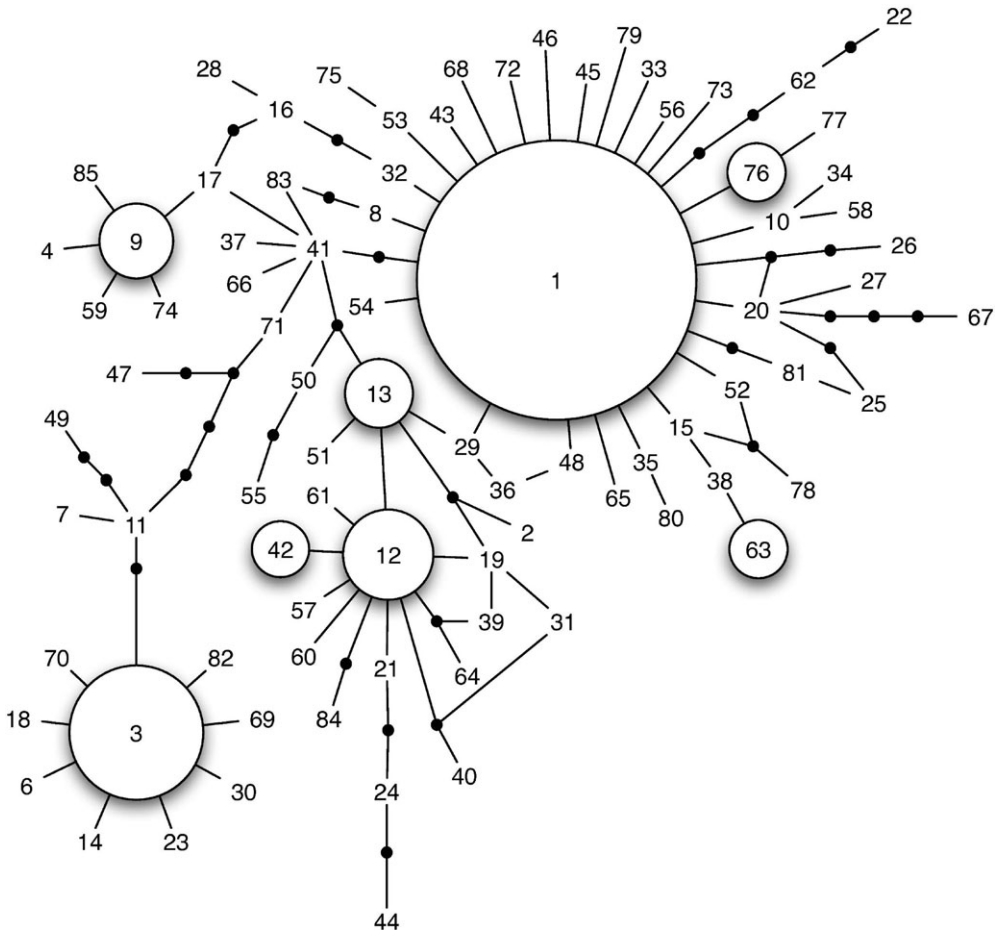

Figure 2. Statistical parsimony network among 85 *M. mercenaria* COI haplotypes. Numbers correspond to haplotypes in Table 1. Haplotypes occurring in frequency >5 are circled, with circle size proportional to frequency. Lines connecting haplotypes represent a difference of a single base pair substitution. Filled circles represent either extinct or unsampled haplotypes.

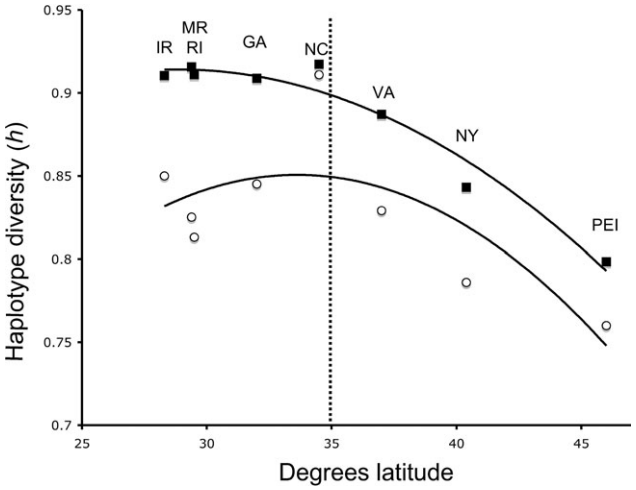

Figure 3. Population diversity vs. latitude for eight Atlantic *M. merceneria* samples. Open circles represent mean pairwise nucleotide differences, squares represent haplotype diversity. The latitudinal position of Cape Hatteras is indicated by the vertical dotted line. Polynomial trend lines are included. Population abbreviations are as in Table 1.

function of latitude (Figure 3), although not in a linear fashion. Samples from north of Cape Hatteras (VA, NY, and PEI) declined in diversity, whereas those from points south of Cape Hatteras plateaued in terms of h, but declined from a peak value of π at NC.

Population differentiation estimated from π (Table 2) showed that following intrapopulation correction, mean pairwise differentiation between populations was low, ranging from effectively zero (13 negative pairwise comparisons; Table 2) to a maximum of 0.069% between GA and PEI. Populations from New River, NC, south through Matanzas River, FL (NC, GA, RI, MR), clustered together on the distance tree, as did Gulf Coast samples (CK, SK) (Figure 4). There was no discernible geographic pattern to the remainder of the locations.

The overall AMOVA results (Table 3) indicated low but significant structure with $\Phi_{SC} = 0.0213$, ($p < 0.002$) ($\Phi_{SC} = 0.0207$, $p < 0.001$ without Gulf coast samples). Pairwise estimates showed significant structure in 16 of 45 comparisons (Table 4). Notably, PEI constituted seven of these significant comparisons. Significant heterogeneity between groups (combining regional samples) was detected only in the "Cape Hatteras" contrast ($\Phi_{CT} = 0.076$, $p = 0.013$), where Group 1 {PEI, NY, VA} differed from the samples from south of Cape Hatteras. Consistent with results from pairwise estimates of differentiation, most of the heterogeneity was attributed to within population variation (representing >90% of the variation across all tests).

Discussion

Our mtDNA survey of *M. mercenaria* revealed a cluster of closely related haplotypes, and no evidence of cryptic evolutionary lineages. Our results indicate fine-scale population structure, despite the lack of phylogeographic signal, based on a significant partition at Cape Hatteras, and a weakly supported partition at Cape Cod.

Although our results could be confounded by transplantation, we do not think that anthropogenic factors have obscured natural genetic partitions. The aquaculture industry for quahogs is relatively young, within the past three decades and primarily the past 15 years, making the widespread dissemination of alien haplotypes unlikely. The northern quahog has a lifespan measured in decades (Peterson, 1986; Jones *et al.*, 1989), although reproductive maturity is reached in less than 3 years (Eversole, 2001), which is also the typical age of harvesting. Further, wild populations typically outnumber aquaculture "populations" by one to two orders of magnitude, with perhaps the exception of the Gulf of Mexico (Fegley, 2001).

The sample from the Gulf of St Lawrence (Prince Edward Island) is significantly different from every sample south of Cape Cod, from New York to Georgia ($\Phi_{SC} = 0.034–0.079$). However, we temper our interpretation of a corresponding biogeographic barrier at Cape Cod with the finding that this break is not supported by AMOVA (Table 3, Group 2). Although the AMOVA for groups north and south of Cape Cod approached significance ($\Phi_{CT} = 0.0323$, $p = 0.1005$), our sampling distribution north of Cape Cod is too limited to draw definitive conclusions. Notably, a recent review of intertidal phylogeographic patterns in the northwestern Atlantic found no consistent pattern to indicate that Cape Cod is an important barrier (Wares, 2002).

Cape Hatteras in North Carolina (35°13′N 75°32′W) is another recognized biogeographic barrier (Calder, 1992; Engle and Summers, 1999), and there is a habitat discontinuity for *M. mercenaria* across the Gulf of Maine (~41°39′N 45°37′W). There were no deep phylogenetic splits detected at these locations, but

Table 2. *Mercenaria mercenaria* population average pairwise differentiation.

	PEI	NY	VA	NC	GA	MR	RI	IR	CK	SK
PEI	***0.7595***	0.8030	0.8220	0.8909	0.8714	0.8162	0.8297	0.8416	0.9152	0.8146
NY	0.0302	***0.7863***	0.8213	0.8875	0.8674	0.8155	0.8308	0.8464	0.9125	0.8180
VA	0.0279	0.0138	***0.8286***	0.8911	0.8750	0.8155	0.8384	0.8554	0.9063	0.8309
NC	0.0557	0.0389	0.0213	***0.9109***	0.8736	0.8664	0.8621	0.8743	0.9414	0.8840
GA	0.0694	0.0519	0.0383	−0.0042	***0.8447***	0.8358	0.8245	0.8390	0.9364	0.8521
MR	0.0300	0.0159	−0.0052	0.0045	0.0070	***0.8129***	0.8065	0.8258	0.9065	0.8178
RI	0.0374	0.0251	0.0115	−0.0060	−0.0105	−0.0126	***0.8251***	0.8217	0.9190	0.8296
IR	0.0366	0.0281	0.0158	−0.0064	−0.0086	−0.0059	−0.0161	***0.8504***	0.9286	0.8437
CK	0.0617	0.0457	0.0183	0.0123	0.0403	0.0263	0.0327	0.0297	***0.9474***	0.9088
SK	0.0158	0.0058	−0.0026	0.0095	0.0106	−0.0077	−0.0021	−0.0006	0.0160	***0.8382***

Above the diagonal is the average number of pairwise differences (π) between populations. Diagonal (italicized and emboldened) values are average π within populations. Below the diagonal are average corrected values of π between populations. Population site codes are as in Table 1.

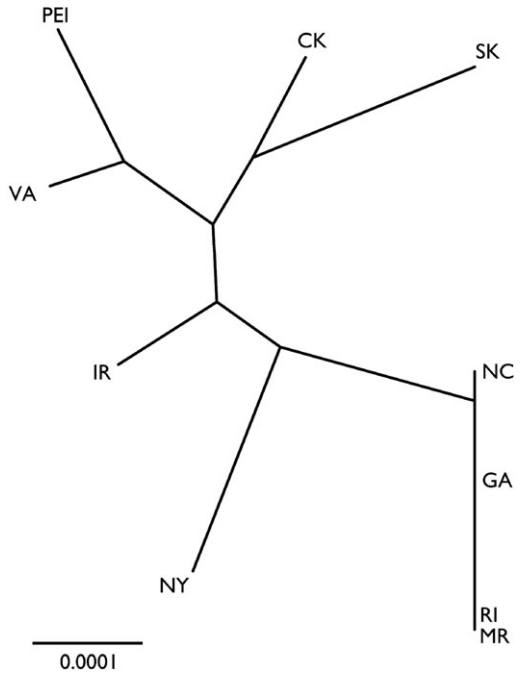

Figure 4. Unrooted population tree based on Kimura two-parameter distances. Abbreviations follow those in Table 1.

Table 3. AMOVA results for among-group components of population genetic variance only

Groups		% of total variance	*p*-value
No barriers			
1	All populations		
1a	{PEI, NY, VA, NC, GA, MR, RI, IR, CK, SK}	2.13	= 0.002
1b	{PEI, NY, VA, NC, GA, MR, RI, IR}	2.07	= 0.001
Putative marine barriers or proposed breaks			
2	Cape Cod		
2a	{PEI} { NY, VA, NC, GA, MR, RI, IR, CK, SK}	3.23	= 0.101
2b	{PEI} { NY, VA, NC, GA, MR, RI, IR}	3.44	= 0.263
3	Cape Hatteras		
3a	{PEI, NY, VA} {NC, GA, MR, RI, IR, CK, SK}	7.63	= 0.013
3b	{PEI, NY, VA} {NC, GA, MR, RI, IR}	9.71	= 0.019
4	Cape Canaveral		
4a	{PEI, NY, VA, NC, GA, MR, RI} {IR, CK, SK}	0.00	= 0.926
4b	{PEI, NY, VA, NC, GA, MR, RI} {IR}	0.00	= 0.860
5	Florida peninsula		
	{PEI, NY, VA, NC, GA, MR, RI, IR} {CK, SK}	0.00	= 0.385

Abbreviations are as in Table 1. Each test was performed with and without putative introduced samples from the Gulf Coast (CK and SK). The last test compares Gulf Coast samples with the Atlantic populations.

population structure was evident at Cape Hatteras. Samples from Cape Hatteras to north Florida did cluster together in the Neighbour-joining analysis, as did the two Gulf Coast samples (Figure 4), but there was no observable structure among the remaining samples. Similar evidence from AMOVA tests also detects a significant but small pattern of differentiation demarcated by Cape Hatteras. However, the influence of ocean currents on patterns of gene flow will depend strongly on species-specific characteristics (Auer, 1987; Gaylord and Gaines, 2000), and it is unclear to what extent currents may be shaping ongoing patterns of gene flow in this species.

Our survey did not detect a phylogeographic break between Atlantic and Gulf of Mexico samples of *M. mercenaria*. Other benthic invertebrates that span this range have demonstrated deep population structure and secondary contact zones near Cape Canaveral (28°27′N 80°31′W), on the east (Atlantic) coast of the Florida Peninsula, where the tropical Gulf Stream moves offshore (Pickard and Emery, 1982; Avise 1992; Sarver *et al.*, 1992; Hare and Avise, 1998). The lack of any Gulf–Atlantic allopatric isolation in *M. mercenaria* is tempered by our limited number and distribution of Gulf Coast samples, and the possibility that our samples represent escapes from local aquaculture. *Mercenaria mercenaria* may exist naturally elsewhere in the Gulf of Mexico, misidentified as the morphologically similar *M. campechiensis*, but this has not been demonstrated (Ó Foighil *et al.*, 1996). Some published reviews included the Gulf of Mexico in the range of *M. mercenaria* (e.g. Eversole, 1987), but an extensive west Florida clam survey failed to report them (Godcharles and Jaap, 1973) and the other reviews terminate the species range in east Florida (Abbott, 1974, 1986; Harte, 2001).

Warm water is known to be a limiting physiological factor for *M. mercenaria* (Grizzle *et al.*, 2001), and the southern range limit, regardless of whether it separately occurs in the Gulf of Mexico, is the Indian River Lagoon on the Atlantic coast of the Florida peninsula (Dillon and Manzi, 1987). The southern tip of the Florida peninsula, therefore, probably represents a barrier to *M. mercenaria*. The eastern oyster (*Crassostrea virginica*) is found around the tip of the peninsula (Cake, 1983), but exhibits phylogeographic structure with a break between Gulf and Atlantic haplotypes at Cape Canaveral. This has been interpreted as reflecting secondary contact between previously isolated Gulf and Atlantic populations of *C. virginica* (Reeb and Avise, 1990; Hare and Avise, 1998). The lack of divergence between Gulf and Atlantic *M. mercenaria*, together with its absence in south Florida, indicates that the population we sampled in the Gulf of Mexico is the product of recent aquaculture introductions.

Physiology may also interact with geography to reduce phylogenetic structure in *M. mercenaria*. The modern latitudinal range of *M. mercenaria* represents ~19° of latitude; it was probably less at the end of the Pleistocene (Pielou, 1992), restricted by cold water to the north and tropical waters in the Strait of Florida to the south. There is a 25°C mean temperature variation across this range, compared with only 10°C over the same latitudes in the eastern Atlantic (Pickard and Emery, 1982). The patchy distribution of *M. mercenaria* north of Cape Cod (Mackenzie *et al.*, 2001) may indicate that recently colonized areas contain populations under selection associated with range expansion (Bernatchez and Wilson, 1998; Phillips and Shine, 2006).

Some sympatric bivalves with deeper phylogeographic structure also have a wider geographic–thermal range. The eastern oyster extends as far north as *M. mercenaria*, but also farther

Table 4. FST values among all populations (below diagonal), and significance (above diagonal).

	PEI	NY	VA	NC	GA	MR	RI	IR	CK	SK
PEI	–	*	*	**	***	+	*	*	**	+
NY	0.0376	–	+	*	**	+	+	*	*	+
VA	0.0340	0.0168	–	*	*	+	+	+	+	+
NC	0.0622	0.0435	0.0237	–	+	+	+	+	+	+
GA	0.0796	0.0598	0.0438	−0.0049	–	+	+	+	*	+
MR	0.0369	0.0196	−0.0064	0.0049	0.0083	–	+	+	+	+
RI	0.0453	0.0303	0.0137	−0.0072	−0.0127	−0.0156	–	+	*	+
IR	0.0433	0.0330	0.0185	−0.0073	−0.0103	−0.0072	−0.0197	–	*	+
CK	0.0712	0.0528	0.0217	0.0135	0.0448	0.0309	0.0371	0.0336	–	+
SK	0.0211	0.0080	−0.0029	0.0093	0.0123	0.0091	−0.0023	−0.0010	0.0170	–

Population site codes are as in Table 1.
+, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

south, around the tip of the Florida peninsula and throughout the Gulf of Mexico (Cake, 1983), and exhibits a clear phylogenetic break in Florida that reflects allopatric isolation and secondary contact following the Pleistocene (Reeb and Avise, 1990). The comparatively narrow tolerances of *M. mercenaria*, for both temperature and salinity (Grizzle *et al.*, 2001), indicates that it may have reduced opportunity to disperse through more severe environments.

Life history traits of *M. mercenaria* may decrease the rate of lineage sorting and, hence, the pace of genetic drift and corresponding post-glacial population structure. Female *M. mercenaria* in the northern part of the range may reproduce at 2 years of age but continue to grow and live for decades, so the average generation time is probably much greater than 2 years (Eversole, 2001). The Holarctic bivalve *Macoma balthica*, in contrast, is a short-lived species that has shown distinct genetic structure among post-glacial habitats (Luttikhuizen *et al.*, 2003).

Our mtDNA survey reveals the characteristic indications of population expansion. Fu's *F*-test demonstrates a significant excess of low-frequency haplotypes, the predicted outcome of population expansion (Rogers and Harpending, 1992), and genetic diversity has a declining trend with increasing latitude, particularly in populations north of Cape Hatteras. This genetic signature of post-glacial dispersal into high latitudes is observed in a variety of coastal organisms, including other molluscs (Cunningham and Collins, 1998; Hellberg *et al.*, 2001; Wares and Cunningham, 2001; Lee and Ó Foighil 2005), and vertebrates (Bowen *et al.*, 1993). Mitochondrial DNA diversity in *M. mercenaria* fits a population growth model, with a skewed haplotype frequency represented by, in this case, one predominant haplotype that is found throughout the sampled range plus numerous rare haplotypes (Rogers and Harpending, 1992). The test for expansion (Fu's *F*) strongly supported this interpretation. A result of such skewed haplotype diversity is a reduced power to detect significant population differentiation (Hauser *et al.*, 2001). Further, the non-linear pattern in π observed south from the midpoint of the range (Figure 3) also indicates that populations towards Georgia and Florida may be younger or affected by more recent bottlenecks (see below) than the Carolina (NC) population, perhaps a consequence of greater variance in environmental characteristics (e.g. water temperature or salinity). This pattern of highest diversity in the centre of the range is also observed in sardine and anchovy in the temperate zone of the Northeast Pacific (Lecomte *et al.*, 2004). An allozyme survey of *M. mercenaria* from Virginia to Massachusetts revealed a lack of difference in allele frequencies across this range (Humphrey, 1981), supporting our interpretation of a single genetic population across the central portion of the range.

Both the weighted mean of pairwise divergence among haplotypes (π) and the relative frequency of haplotypes (without consideration of their evolutionary relationships, *h*) provide a basis to infer patterns and events affecting past population demographics. Grant and Bowen (1998) defined four demographic categories based on the relationship between *h* and π. Prolonged bottlenecks are expected to have an impact on both *h* and π. Brief bottlenecks should result in some loss of haplotypes (low *h*) but have relatively little impact on π. Rapid population growth from an ancestral population with a low N_e (effective population size) would result in high *h* and low π. Finally, large, stable N_e or admixed populations should have high values in both diversity measures. All samples analysed here (Figure 3) had high values of h (>0.5) and low π ($<0.01\%$), which we interpret as reflecting rapid population growth from a small ancestral population. This scenario is realistic in a species with high reproductive variance and sensitivity to environmental conditions.

In conclusion, the current evidence suggests that the entire range of *M. mercenaria* represents a single evolutionary unit that shared a common Pleistocene refugial area located (based on diversity indices) around the Carolinas. Expansion northwards is evident by the reduced diversity in mtDNA haplotypic data, mismatch distribution, and excess of low frequency haplotypes. The significant differentiation at Cape Hatteras and the tentative indications of a barrier at Cape Cod are likely a result of oceanographic conditions, range expansion, and modest dispersal ability. From a management perspective, our findings should be tempered by the knowledge that we are examining only the mitochondrial genome, and that putatively neutral genes are not a good indicator of adaptive variation (Reed and Frankham, 2001).

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