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An investigation of genetic population structure in blue crabs, *Callinectes sapidus*, using nuclear gene sequences

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Abstract Spatial and temporal genetic heterogeneity in the sequences of nuclear protein-coding genes were investigated in populations of the blue crab, *Callinectes sapidus*. Haplotype frequencies of these novel markers were determined for adult crabs collected along 300 km in the northern Gulf of Mexico in two different years (2010 and 2011), as well as for megalopal recruits collected in 2010. Tests of genetic differentiation among all locations and between locations spanning known genetic breaks in other species were conducted. In addition, samples from distant locations within the range of *C. sapidus* were used to assess genetic divergence on a broader geographic scale. Significant between-year differences were found for adults at one location and near significant spatial differentiation was found across northern Gulf of Mexico locations in 2010. These results suggest that although the large population sizes and meroplanktonic life history of blue crabs promote widespread gene flow on a regional scale, genetic composition can change over just one year. Substantial divergence between the northern Gulf of Mexico and Venezuela suggests the possibility that temporal shifts in haplotype frequencies could result from variation in the rate of immigration from genetically distinct source populations. The possible effects of the Deepwater Horizon Oil Spill and attendant fisheries closures during the sampling phase of this study are also considered.

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Introduction

Understanding the factors that determine connectivity among locations is essential for the management and conservation of marine populations (Gaines et al. 2003; Shaklee and Bentzen 1998). In particular, identification of stock boundaries for fishery species and the design of marine reserves for threatened species require reliable information about the number of migrants being exchanged among locations. This knowledge can also be used to predict how effectively populations decimated by natural or human pressures can be replenished by surrounding populations. For marine invertebrates, assessments of larval dispersal must often rely on genetic markers when more direct approaches, such as physical tagging, are impractical (Thorrold et al. 2002; but see Gelpi et al. 2013).

Marine species with offshore pelagic larvae often show little or no genetic differentiation over large distances (Shaklee and Bentzen 1998). This is generally attributed to the widespread gene flow that results from the transport and mixing of larvae by ocean currents. If even slight genetic structure is observed, this is usually interpreted as evidence for barriers to larval dispersal (Palumbi 1994). Several mechanisms have the potential to act as barriers: larval behavior that favors retention in natal habitats (Morgan and Fisher 2010; Cronin 1982; Swearer et al. 2002), physical oceanographic barriers that limit advection (Galarza et al. 2009; Waters and Roy 2004), or larval mortality that occurs during dispersal or after settlement at a new location (Johnson and Black 1982; Hilbish 1985; Treml et al. 2012).

The blue crab, *Callinectes sapidus*, is an important recreational and commercial fishery species, with US commercial landings in 2011 valued at over $181 million (NOAA 2013). The life history of this species favors dispersal and mixing of larvae from widespread sources. Female blue

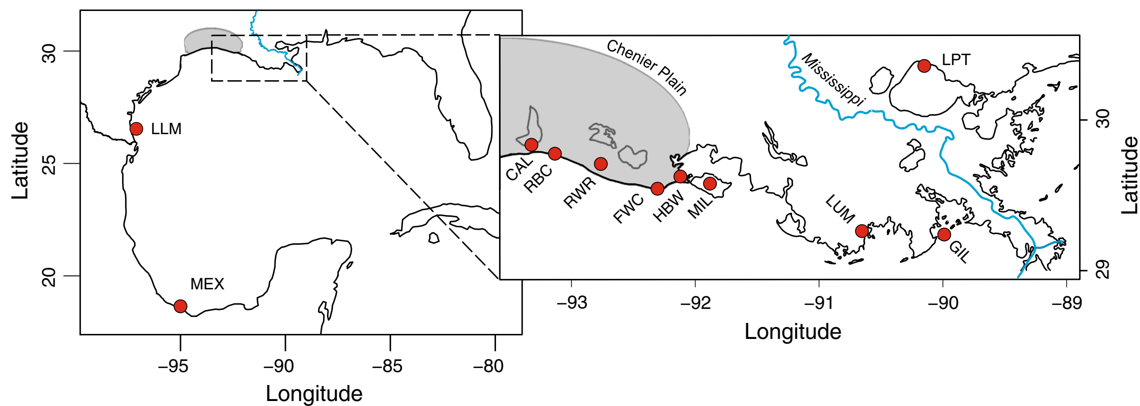

Fig. 1 Sampling locations in the Gulf of Mexico, with an *inset* detailing the sampling sites along the Louisiana Coast. Location abbreviations are defined in Tables 1, 2

crabs mate only once and use stored sperm to fertilize up to 18 broods of eggs over the course of their 3.5-year life span (Hines et al. 2003; Millikin and Williams 1984; Dickinson et al. 2006). Fecundity is very high; each brood can contain up to 3.2 million eggs (Hsueh et al. 1993), but clutch volume, egg size, and hatching success decreases significantly over successive broods (Graham et al. 2012; Darnell et al. 2009, 2010). During brood development, ovigerous females migrate from the brackish upper regions of estuaries to saline coastal waters (Hench et al. 2004; Forward et al. 2005) where larvae are eventually released at high or ebb tide (Hench et al. 2004). This timing of larval release is thought to maximize transport of larvae to offshore waters.

The pelagic larval duration (PLD) of blue crabs ranges from 31 to 49 days and is followed by a post-larval megalopal phase lasting 6–20 days (Costlow and Bookhout 1959). Variation in PLD and the length of the megalopal phase largely depends on water temperature and salinity (Costlow and Bookhout 1959; Sulkin and Van Heukelem 1986). Following development in offshore waters, megalopae return to estuaries via wind forcing, surface currents, and tidal transport (Rabalais et al. 1995; Morgan et al. 1996; Epifanio and Garvine 2001; Perry et al. 1995) where they settle in areas with submerged vegetation (Heck et al. 2001) and molt to a first crab stage within a few days.

Surface currents in the Gulf of Mexico (GOM) provide a mechanism for dispersal of pelagic larvae. Strong forcing by easterly winds over the Texas–Louisiana shelf creates long-shore currents with mean velocities around 25 cm s^{-1} on the inner shelf that flow westward and southward along the Texas Coast to approximately 27°N where the currents are deflected offshore to deeper waters (Ohlmann and Niiler 2005; Ohlmann et al. 2001). Wind reversal from April through July turns the current on the inner shelf eastward (Ohlmann and Niiler 2005). Currents on the outer shelf are more variable, but generally flow eastward in response to anticyclonic activity on the shelf edge (Nowlin et al. 2005). These patterns suggest larvae released along the Texas–Louisiana shelf should disperse westward along the shelf for some time, but could be brought back eastward by outer shore or inshore summer currents. Additionally, these eastward flowing currents could bring larvae to the Louisiana Coast from western and southern GOM locales. Oceanographic connectivity between the Texas–Louisiana shelf and the northeastern GOM is generally limited, but strong wind events, storms (Ohlmann and Niiler 2001), and eddy detachment from the Loop Current (Johnson and Perry 1999) can create occasional westward flows connecting the two regions.

Surveys of genetic markers have revealed that for some species in the GOM, there is spatial population structure despite the potential for widespread planktonic dispersal (e.g., Varney et al. 2009; Bilodeau et al. 2005; Drumm and Kreiser 2012; Neigel 2009). For example, two prominent genetic breaks have been identified on the east and west coasts of the GOM at the boundary between the Carolinian Marine Province of the northern GOM and the tropical waters of the southern GOM (Neigel 2009). Additional biogeographic boundaries at the mouth of the Mississippi River and the Chenier Plain to the west (Fig. 1) have also been associated with genetic breaks for marine species (Bilodeau et al. 2005; Herke and Foltz 2002). Unfortunately, past surveys of genetic markers for blue crabs within the GOM present a confusing picture, with evidence for both genetic homogeneity across the northern GOM as well as pronounced genetic heterogeneity on a much smaller scale. Reconciliation of these disparate findings is complicated by differences among studies in statistical power, mode of marker inheritance, and the potential for natural selection to influence the markers.

Berthelemy-Okazaki and Okazaki (1997) found no significant genetic differentiation at nine allozyme loci for

blue crabs collected from Louisiana, Alabama, and Texas, although this study had relatively little statistical power to detect differences. Genetic homogeneity was also found for 15 out of 19 polymorphic allozyme loci (McMillen-Jackson et al. 1994) and mitochondrial DNA (McMillen-Jackson and Bert 2004) surveyed in blue crabs that were collected along the US Atlantic and GOM coasts. In contrast, significant spatial heterogeneity in allele frequencies at three allozyme loci was documented in the eastern GOM, as well as temporal heterogeneity at an esterase locus (McMillen-Jackson et al. 1994). Even more striking are the findings of Kordos and Burton (1993) that include significant differentiation at three allozyme loci among adults collected along a 600 km stretch of the Texas Coast. Furthermore, significant temporal differences were also found among samples of megalopae collected at the same location over several months, with two loci showing drastic shifts in allele frequencies at multiple locations (Kordos and Burton 1993). Possible explanations offered for these anomalous patterns included seasonal influxes from hypothetical source populations, which would be more genetically diverse than any known for blue crabs, and post-settlement selection acting on multiple allozyme loci in early life stages (Kordos and Burton 1993).

The aim of the present study was to provide a new assessment of genetic structure in blue crabs in the northern GOM (nGOM) by surveying sequences of five nuclear protein-coding genes. This study is also intended as a baseline component of a long-term study of the population biology of the blue crab in the nGOM. The partial gene sequences used in this study are novel genetic markers for blue crabs, selected because they: (1) are highly polymorphic, providing adequate statistical power to detect relatively small differences among samples; (2) are from multiple independently segregating loci, which contributes to statistical power and reduces stochastic variance in estimates of population parameters; (3) differ by single nucleotide substitutions, which can be scored objectively and fit standard population genetic models; (4) encode proteins with known physiological roles; and (5) with one exception, are amplified by exonic primers, which reduces the chance of null alleles.

We have no a priori information on the selective neutrality of the genes we chose. Thus, there is the potential for these markers to be influenced by natural selection, either directly or indirectly by selection on linked regions. However, this caveat is not unique to these markers. Mitochondrial genomes can experience strong selection, either directly or through hitchhiking effects from other maternally inherited factors (Bazin et al. 2006; Meiklejohn et al. 2007). Similarly, allozymes (Koehn et al. 1980), microsatellites (Larsson et al. 2007; St-Onge et al. 2013), and single nucleotide polymorphisms (SNPs) (Therkildsen et al. 2013; Bradbury et al. 2010) have also been found to show patterns that are consistent with selection in marine populations. In contrast to most other markers, however, sequences of known protein-coding genes offer a biological context in which to interpret departures from neutral expectations should they exist.

The main objectives of this study are intended to address questions raised in previous studies by testing for: (1) spatial genetic differentiation in adults collected from different estuaries, (2) temporal genetic changes in adults over 2 years, and (3) changes in allele frequencies between the megalopal and adult life stages of cohorts collected at four locations. Nine sampling locations were chosen to represent different estuarine systems and a range of habitats. These locations also span two well-known genetic breaks for marine species in the nGOM: the Chenier Plain in the northwestern GOM and the Mississippi River in the central GOM (Fig. 1). The sampling design, with a concentration of sites along the coast of Louisiana, large sample sizes, and good statistical power, provides a sensitive test for any pattern resembling these genetic breaks in blue crabs. Gene sequences of blue crabs from southern Texas, Mexico, and Venezuela were also determined to assess the possibility that populations elsewhere in the GOM or Caribbean could act as sources of genetically divergent larvae.

Materials and methods

Sample collection

Adult crabs were collected at up to nine locations along the Louisiana Coast (Fig. 1; Table 1) from May to July in 2010 and 2011. Crabs were collected with baited hand lines, hoop nets, and recreational vinyl-coated wire mesh traps. One walking leg was removed from each crab and stored in 95 % ethanol prior to DNA extraction. Megalopae were also collected in 2010 at four locations for intracohort comparisons with the adult crabs collected at the same locations the following year (Table 1). All megalopae collections were done using passive larval samplers, of a design adapted from Metcalf et al. (1995), which were deployed for up to 2 weeks. Collectors were rinsed in freshwater, and megalopae were transferred from the rinse water to 95 % ethanol. Because megalopae molt to a first crab stage within a day of settling, we considered all megalopae removed at the same time from a collector to represent a single settlement event. Whenever possible, samples were stored at 4 °C.

Adult and juvenile blue crabs were collected in Lower Laguna Madre (LLM), Texas, during the summer of 2010 and stored in 95 % ethanol (Fig. 1; Table 2). Adult and juvenile specimens of blue crabs from the University of Louisiana at Lafayette Zoological (ULLZ) Collection that

Table 1 Blue crab sampling locations and sample sizes of adults (A) and megalopae (M) collected in Louisiana in 2010 and 2011

Abbreviation	Location name	Latitude	Longitude	2010		2011
				A	M	A
CAL	Calcasieu Lake	29.8382	−93.3206	24	0	0
RBC	Rutherford Beach Culvert	29.7789	−93.1326	20	0	0
RWR	Rockefeller Wildlife Refuge	29.7121	−92.7656	17	26	16
FWC	Freshwater City Locks	29.5520	−92.3055	24	24	24
HBW	Hog Bayou Weir	29.6202	−92.1163	31	0	0
MIL	Marsh Island	29.5777	−91.8842	24	0	0
LUM	LUMCON Marine Lab	29.2540	−90.6639	27	24	24
LPT	Lake Pontchartrain	30.3617	−90.1664	32	0	0
GIL	Grand Isle	29.2394	−90.0020	53	48	23
			Totals	253	122	87

Table 2 Blue crab sampling locations and sample sizes for adult (A), juvenile (J), and unknown (U) life-stage crabs that were collected outside of Louisiana

Abbreviation	Location	Latitude	Longitude	A	J	U	Total
LLM	Lower Laguna Madre, Texas, USA[a]	26.2336	−97.1983	4	13	0	17
MEX	Veracruz, Mexico[b]	*18.5302*	*−95.0262*	0	0	5	5
VEN	Zulia, Venezuela[c]	*10.9476*	*−71.1931*	4	4	6	14
			Totals	8	17	11	36

Coordinates in italics indicate approximate locations based on the University of Louisiana at Lafayette Zoological (ULLZ) Collection specimen information

[a] Collected from May–June in 2010

[b] ULLZ specimens collected in April of 2000

[c] ULLZ specimens collected from 1993 to 2003

were collected in Mexico (MEX) and Venezuela from 1993 to 2003 were also included in this study (Fig. 1; Table 2).

Marker selection

Sequences from expressed sequence tag (EST) libraries representing 10,563 ESTs from blue crabs (Coblentz et al. 2006) were used to target nuclear protein-coding genes for PCR and sequencing. The ESTs were assembled into contigs using the computer program CAP3 (Huang and Madan 1999). The consensus sequence of each contig was queried in the NCBI non-redundant nucleotide database (GenBank Release 183, April 2011) using the BLASTN 2.2.25 + algorithm (Zhang et al. 2000) with a gap penalty of zero and a gap extension penalty of 2.5. Database matches with an E value $<10^{-5}$ were considered significant and used for contig annotation. Five genes were chosen for their putative physiological roles—two stress response genes: heat shock protein 70 (*hsp*) and trehalose 6-phosphate synthase (*tps*); two energy metabolism related genes: ATP-synthase subunit 9 (*atps*) and ATP/ADP translocase (*ant*); and one housekeeping gene: ribosomal protein L12 (*rpl*). All PCR primers were designed with the online program Primer3 (Rozen and Skaletsky 2000) using alignments of the EST contig consensus sequence and the BLAST match for the crustacean species with the best E value and the highest maximum identity score (Table 3).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted using either the PUREGENE® DNA Purification Kit protocol for DNA isolation from marine invertebrate tissue (Gentra Systems, Inc.) or NucleoSpin® 96 Tissue kits (Macherey–Nagel) adapted for use on an automated liquid handling workstation (epMotion 5075 TMX, Eppendorf). DNA was extracted from entire megalopae or approximately 20 mg of leg muscle from adult crabs. DNA was quantified with a NanoDrop 1000 (ThermoFisher Scientific) and checked for degradation on ethidium bromide-stained agarose gels. The polymerase chain reaction (PCR) was used to amplify portions of each of the five nuclear protein-coding genes in 15 µl reactions containing: 1.5 µl (10×) AmpliTaq Gold® PCR buffer (Applied Biosystems), 1.5 µl (25 mM) $MgCl_2$, 1.2 µl (10 mM) dNTPs, 0.9 µl (20 µM) of each forward and reverse primer, 0.6 units of AmpliTaq® Gold (Applied

Table 3 Sequences and annealing temperatures (T_A) of forward (F) and reverse (R) primers used for PCR amplification, as well as the reference sequences used for primer design

Primer name	Direction	Primer sequence (5′–3′)	GenBank accession (reference species)	Position in reference sequence	Product size (bp)	T_A (°C)
ATP/ADP translocase (*ant*)						
ANT3F	F	CTT GCT GTA TTG GAA CCC TTT	EF077712 (*Marsupenaeus japonicus*)	413–898	486[a]	65
ANT4R	R	GAG CCT TTG AGC AAC TGA CC				
ATP-synthase subunit 9 (*atps*)						
564.363F1	F	CTG GTG CTG GTA TTG GGT CT	HM217802 (*Scylla paramamosain*)	103–422	320	60
564.363R1	R	GAA TAC CTC GCC TCA CCA AG				
Trehalose 6-phosphate synthase (*tps*)						
TPS5F	F	TTG CTG CTT GAC TAC GAT GG	EU679406 (*Callinectes sapidus*)	1,471–1,957	487	60
TPS4R	R	GGC CTT TGT TCC ATG TGA CT				
Heat shock protein 70 (*hsp*)						
HSP2F	F	CAT CAA ACG TAA CAC CAC CAT C	DQ663760 (*Callinectes sapidus*)	1,332–1,931	600	68
HSP2R	R	GCT TGG TAC ATC TTG GTG ATG A				
Ribosomal protein L12 (*rpl*)						
RPL12F1	F	TCC CCC AAG AAG GTT GGT	FJ774832.1 (*Scylla paramamosain*)	221–411	191	51.4
RPL12R1	R	TTG TGC TTG ATG TGC TTA ACC T				
16S rRNA						
16ar[b]	F	CGC CTG TTT ATC AAA AAC AT	AY363392 (*Callinectes sapidus*)			
16br[b]	R	CCG GTC TGA ACT CAG ATC ACG T		3,095–3,655	561	55

[a] Includes 4 indels (*C. sapidus* product size is 482)

[b] Primers from Palumbi et al. (1991)

Biosystems) and 5–25 ng of template DNA. PCRs were carried out on a Bio-Rad iCycler™ with the following profile: 10 min at 94 °C; followed by 35 cycles of 20 s at 94 °C, 20 s at 51.4–68 °C, 30 s at 70 °C; 5 min at 70 °C; and held at 4 °C. Primer sequences and annealing temperatures for all loci are shown in Table 3.

Because previous studies reported difficulty in distinguishing megalopae of *C. sapidus* from those of congeners (Stuck and Perry 1981), identification of all megalopae was verified to species with mitochondrial 16S ribosomal DNA sequences; this gene was chosen because sequences from reference specimens with vouchers are available for every species of *Callinectes* reported from the Gulf of Mexico (Robles et al. 2007). PCR amplification of a portion of the 16S gene followed the above protocol, but with the following thermocycling profile: 10 min at 95 °C; followed by 40 cycles of 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C; 2 min at 72 °C; and held at 4 °C. Sequences for the 16S primers and amplification details are shown in Table 3.

Prior to sequencing, 3 µl of each PCR was electrophoresed in an ethidium bromide-stained agarose gel to confirm amplification of a single product. The remainder was treated with 0.1 µl (20 U/µl) exonuclease I (New England Biolabs, Inc.), 0.3 µl (5 U/µl) Antarctic Phosphatase (New England Biolabs, Inc.), and 6.6 µl milli-Q filtered water, then heated to 37 °C for 1 h 15 min, 95 °C for 5 min, and held at 4 °C. Cycle sequencing reactions were performed in 10 µl total volume reactions with 4.5 µl milli-Q filtered water, 2.5 µl (5X) sequencing buffer [0.4 M Tris–HCl pH 9, 10 µM $MgCl_2$], 2 µl (0.8 µM) primer, and 0.5 µl BigDye® Terminator v.1.1 (Applied Biosystems). Thermocycling conditions followed Platt et al. (2007). Cycle sequencing products were cleaned by standard ethanol precipitation, rehydrated in 20 µl HiDi Formamide (Applied Biosystems), and denatured at 95 °C for 3 min, then held at 4 °C. All sequencing reactions were run on an ABI 310 Genetic Analyzer (Applied Biosystems), and basecalls were made with Sequencing Analysis software version 5.2 (Applied Biosystems) using the KB basecaller. Sequences were aligned and edited in the SeqMan module of DNASTAR Lasergene software version 8.0.2 (DNASTAR, Inc.). Haplotypic phase of edited sequences was determined using the Bayesian method implemented in PHASE v.2.1 (Stephens and Donnelly 2003; Stephens et al. 2001), which has been shown to be an accurate and robust method for reconstructing haplotypes from population genetic data (Calderón and Turon 2010; Harrigan et al. 2008). A total of 1,689 bp of haplotype-phased sequence data, representing portions of five loci ranging from 191 to 489 bp in length (Table 4), was used for subsequent analysis.

Table 4 Sequence and haplotype summary information for each locus

	Sequence Length	S	SS	NS	*h*	*h* (*NS*)
ant	414	34[a]	32	3	54	3
atps	227	29[a]	31	0	43	0
hsp	489	30	18	12	33	15
rpl	191	11[a]	12	1	23	1
tps	368	23[a]	19	5	20	4

S number of segregating sites, *SS* number of synonymous substitutions, *NS* number of nonsynonymous substitutions, *h* number of haplotypes, *h* (NS) number of haplotypes with ≥1 nonsynonymous substitution

[a] ≥1 segregating sites with either >2 alleles or both an *SS* and an *NS* allele

Data analysis

Summary statistics including the number of haplotypes (*h*), segregating sites (*S*), synonymous mutations (*SS*), and nonsynonymous (amino-acid altering) mutations (*NS*) were calculated for the entire sequence data set using DnaSP v.5.10.01 (Librado and Rozas 2009). Unless otherwise noted, all other data analyses were done in GENEPOP v.4.1.1 (Rousset 2008). Basic summary statistics were calculated separately for the 2010 and 2011 adult and 2010 megalopae data sets. The number of haplotypes (*h*), or alleles, and genotypes was calculated, as well as the haplotype frequencies and the observed (H_O) and expected (H_E) heterozygosities for each locus and location. Exact tests of Hardy–Weinberg Equilibrium (HWE) were done by the method of Guo and Thompson (1992) with 20 batches of 5,000 iterations each, and all loci and locations were tested for heterozygote excess and deficit following Rousset and Raymond (1995). All locus pairs were tested for linkage disequilibrium at each location and across all locations, and the inbreeding coefficient (F_{IS}) was estimated based on Weir and Cockerham (1984).

Exact tests of genetic differentiation based on Raymond and Rousset (1995) were used to test for differences in haplotype frequencies for each locus across locations, years, and life stages. Whenever necessary, a standard Bonferroni correction (Rice 1989) was used to determine the significance of multiple tests. Fisher's combined probability test was used for comparisons using multilocus tests with all loci combined. Within-year genetic differentiation was tested separately for the 2010 adult and megalopae data sets and the 2011 adult data set across locations. For 2010, genetic differentiation was tested among all nine locations, as well as among the four locations that were sampled in both years (RWR, FWC, LUM, and GIL). Megalopae collected in 2010 were also pooled across locations and compared with the pooled 2010 adults.

Between-year genetic differentiation among adults was tested at each location individually for the four locations sampled in both years (RWR, FWC, LUM, and GIL) and by comparing the adults pooled across locations for each year. To investigate changes in haplotype frequencies within cohorts, the 2010 megalopae collected at each location (RWR, FWC, LUM, and GIL) were compared with the adults collected at the same locations in 2011. In addition, all megalopae collected in 2010 were pooled and compared with all the adults collected in 2011.

To test for genetic differentiation across the Mississippi River, all locations to the west of the Mississippi River (GIL, LUM, MIL, HBW, FWC, RWR, RBC, CAL) were pooled and compared with LPT. Similarly, locations to the east of the Chenier Plain (LPT, GIL, LUM, MIL) were pooled and compared with LLM to test for a genetic break across this geologic boundary.

Isolation by distance was evaluated for the 2010 Louisiana adult crabs by a Mantel test following Rousset (1997) using the length of coastline between sampling sites and pairwise F_{ST} calculated across all loci. The significance of the Mantel test was determined from the regression of $F_{ST}/(1 - F_{ST})$ on the coastline distance in km between sites.

For each locus, an analysis of molecular variance (AMOVA) was used to estimate divergence among samples collected in Louisiana, Texas, Mexico, and Venezuela. Pairwise estimates of F_{ST} were used to compare all pairs of locations. The adult crabs and megalopae collected in Louisiana were separated for all comparisons with Texas, Mexico and Venezuela. All AMOVA and pairwise F_{ST} tests were run in Arlequin v.3.5.1.2 (Excoffier et al. 2005) using 10,000 permutations.

A power analysis was done using the program POWSIM (Ryman and Palm 2006) to evaluate the power of our data set to detect divergence among nine simulated populations for a range of differentiation levels ($F_{ST} = 0.0001$–0.01). For comparison with our study, power analyses were also run to determine the statistical power of two previously published allozyme data sets that showed significant differentiation among adult blue crabs in the nGOM. These data sets included: three allozyme loci surveyed across eight locations along 600 km of the Texas Coast (Kordos and Burton 1993), and 17 allozyme loci surveyed across eight

locations throughout the nGOM (McMillen-Jackson et al. 1994). Additionally, a power analysis was run for our data set that included non-Louisiana samples to assess how the reduced and uneven sample sizes affected statistical power. All POWSIM analyses included 100 batches of 1,000 iterations and a burn in of 1,000.

Results

Sequence Characteristics

High-quality sequences were obtained for all loci; there was no evidence of multiple products for any of the primer pairs, and all sequences were aligned without indels. Trimmed alignments ranged in length from 191 (*rpl*) to 489 (*hsp*) bases and consisted entirely of coding region, with the exception of *atps*, which includes 100 bases of the 3′ UTR region. These alignments were sufficiently long to include a minimum of 11 segregating sites and distinguish at least 20 haplotypes at each locus (Table 4), with observed heterozygosity at samples from specific locations (Tables 5, 6, 7) ranging from 0.063 (*tps* at MIL for 2011 adults) to 1 (*rpl* at RBC for 2010 adults).

Nonsynonymous substitutions were found at every locus except *atps* (Table 4). In most cases, haplotypes with nonsynonymous substitutions occurred at low frequencies in one or a few locations (Supplementary Tables 1, 2), but the frequencies of haplotype *tps_5* were drastically different between Venezuela (0.893), and all other locations (range 0.002–0.2) (Supplementary Table 2). All haplotypes for *ant*, *atps*, *hsp,* and *tps* have been submitted to GenBank (Accessions: KC886426-KC886589). Because the sequences of *rpl* are shorter than the 200 bp minimum required for GenBank, all *rpl* haplotypes were submitted to the European Nucleotide Archive (HG530328-HG530352).

Departures from Hardy–Weinberg expectations

When all loci and locations were pooled, significant deviations from HWE were detected in both the 2010 (χ^2_{88} = "infinity," $p < 0.0001$) and 2011 (χ^2_{38} = "infinity," $p < 0.0001$) adult data sets, but not the megalopae data set ($\chi^2_{40} = 44.9$, $p = 0.275$). When loci were evaluated individually at each location, *ant* showed significant deviations from HWE in samples of adults from several locations for both years (Tables 5, 7), but not for megalopae (Table 6). All of the significant HWE deviations shown by *ant* were due to heterozygote deficits. Because significant heterozygote deficits are often caused by null alleles, the frequencies of hypothetical null alleles were estimated for each location and year in GENEPOP v.4.1.1 (Rousset 2008) using the expectation maximization (EM) algorithm developed by Dempster et al. (1977). This estimation method has been shown to perform better than other estimation methods (Chapuis and Estoup 2007). The estimates of null allele frequencies for *ant* were considerably higher than those at other loci (Tables 5, 6, 7), and the highest estimates for *ant* corresponded to locations with significant heterozygote deficiencies (Tables 5, 7). Because the presence of null alleles can introduce bias into tests of population genetic differentiation (Chapuis and Estoup 2007) and other population genetic analyses (Dakin and Avise 2004), *ant* was excluded from subsequent power analyses and tests of genetic differentiation. After removing *ant* from the multilocus data sets, no significant departures from HWE were found for samples of adults from 2010 ($\chi^2_{72} = 63.7$, $p = 0.7475$) or 2011 ($\chi^2_{32} = 34.5$, $p = 0.3484$). There was no evidence of linkage disequilibrium for any pair of loci in either year.

Power analyses

A power analysis showed that variation at the four loci (*atps*, *tps*, *hsp*, and *rpl*) provided sufficient statistical power to detect small levels of divergence (e.g., 1.0 at $F_{ST} = 0.01$) across nine locations and greater power than either of the allozyme data sets that were previously used to detect significant genetic differentiation among blue crabs in the nGOM (Supplementary Fig. 1). Comparison with a published power analysis of allozyme and microsatellite data sets, employed for other marine species (Ryman and Palm 2006), shows our data set has higher or equivalent power across the same range of F_{ST} values.

A separate power analysis was run to evaluate the power of the same four loci to detect significant differentiation among simulated populations with the low and uneven sample sizes of the non-Louisiana locations used in this study. The results of this analysis showed the statistical power was very high for comparisons with Louisiana samples (e.g., 0.94 when $F_{ST} = 0.005$ and 0.99 when $F_{ST} = 0.01$).

Temporal and spatial heterogeneity

As with the power analyses, tests of temporal and spatial heterogeneity were based only on the four loci (*atps*, *tps*, *hsp*, and *rpl*) that did not exhibit significant deviation from HWE. A significant temporal genetic difference was found between the two sampling years when adults from all four locations that were sampled in both years (RWR, FWC, LUM, and GIL) were pooled and compared ($\chi^2_8 = 27.5$, $p = 0.0006$). Three of the four loci showed significant between-year differentiation at $\alpha = 0.05$, but only haplotype frequencies at *rpl* were significantly different between years (Table 8) following a Bonferroni correction ($\alpha = 0.0063$). When locations were tested individually,

Table 5 Genetic diversity statistics for partial sequences of five nuclear loci from adult *C. sapidus* collected in 2010

	CAL	RBC	RWR	FWC	HBW	MIL	LUM	GIL	LPT
ant									
N	24	20	17	24	24	24	23	33	24
h	10	12	10	9	12	16	10	10	10
H_O	0.375	0.500	0.529	0.375	0.708	0.542	0.522	0.576	0.333
H_E	0.724	0.736	0.786	0.774	0.805	0.839	0.779	0.681	0.814
F_{IS}	0.488*	0.326	0.333	0.521*	0.122	0.359	0.335	0.156	0.596*
R	0.205	0.134	0.146	0.234	0.048	0.158	0.131	0.089	0.270
atps									
N	24	20	17	24	31	24	24	44	32
h	12	11	8	10	11	8	10	13	11
H_O	0.750	0.650	0.706	0.750	0.645	0.542	0.792	0.636	0.531
H_E	0.736	0.697	0.610	0.717	0.604	0.607	0.768	0.679	0.540
F_{IS}	−0.020	0.070	−0.164	−0.047	−0.070	0.110	−0.032	0.064	0.016
R	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
tps									
N	24	20	17	24	24	24	27	41	24
h	3	3	4	6	7	4	3	4	4
H_O	0.417	0.200	0.353	0.375	0.375	0.167	0.296	0.220	0.250
H_E	0.345	0.268	0.316	0.422	0.423	0.198	0.360	0.202	0.268
F_{IS}	−0.214	0.259	−0.123	0.114	0.115	0.160	0.179	−0.086	0.068
R	0.000	0.085	0.000	0.025	0.025	0.097	0.051	0.000	0.000
hsp									
N	23	20	17	24	24	24	26	34	24
h	10	8	8	9	7	7	11	11	12
H_O	0.783	0.550	0.765	0.667	0.708	0.833	0.769	0.618	0.750
H_E	0.811	0.606	0.759	0.730	0.702	0.720	0.836	0.748	0.762
F_{IS}	0.035	0.095	−0.007	0.088	−0.009	−0.162	0.082	0.176	0.016
R	0.031	0.017	0.000	0.000	0.000	0.016	0.063	0.022	0.000
rpl									
N	23	20	17	24	24	24	25	32	24
h	9	8	7	8	10	12	9	10	11
H_O	0.565	1.000	0.706	0.958	0.875	0.792	0.880	0.875	0.708
H_E	0.814	0.873	0.820	0.831	0.849	0.822	0.849	0.834	0.835
F_{IS}	0.310	−0.150	0.143	−0.158	−0.031	0.037	−0.037	−0.050	0.155
R	0.156	0.000	0.071	0.000	0.022	0.015	0.000	0.000	0.053

Location abbreviations are defined in Table 1

N number of genotypes, *h* number of haplotypes, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient based on Weir and Cockerham (1984), *R* estimated null allele frequency

* Significant F_{IS} values after a standard Bonferroni correction ($p < 0.001$)

samples from LUM showed significant differences between years for the Bonferroni-corrected α of 0.0063 (Table 8). Without LUM, the between-year comparison of pooled locations would not be significant ($p = 0.0693$) (Table 8).

Tests for heterogeneity among locations were conducted separately for 2010 and 2011 samples of adult blue crabs. Fewer tests were conducted for 2011 because only four locations (RWR, FWC, LUM, and GIL) were sampled in that year. Genetic differentiation among all nine Louisiana locations in 2010 was nearly significant for *atps*, *hsp*, and for all loci combined ($\chi^2_8 = 15.3$, $p = 0.0534$) at $\alpha = 0.05$ (Table 8). Tests for an isolation-by-distance pattern sometimes reveal significant genetic structure when comparisons among populations do not (Palumbi 2003). However, no significant pattern of isolation by distance was found along the Louisiana Coast for samples of adult blue crabs collected in 2010 ($p = 0.4390$). Despite the presence of genetic breaks in the nGOM for other marine species, no significant differences were detected for adult blue crabs collected in 2010 across either the present day mouth of the Mississippi River ($\chi^2_8 = 5.0$, $p = 0.7565$) or the Chenier Plain ($\chi^2_8 = 14.9$, $p = 0.0615$), a relict of the Mississippi River's previous mouth location (Penland and Suter 1989). Finally, no significant heterogeneity was found among samples from either year for the four locations (RWR, FWC, LUM, and GIL) that were sampled in both 2010 and 2011 (Table 8).

Table 6 Genetic diversity statistics for partial sequences of five nuclear loci from megalopae of *C. sapidus* collected in 2010

	RWR	FWC	LUM	GIL
ant				
N	26	24	24	25
h	7	9	9	9
H_O	0.385	0.542	0.542	0.520
H_E	0.603	0.590	0.733	0.654
F_{IS}	0.366	0.083	0.265	0.208
R	0.148	0.056	0.092	0.094
atps				
N	26	24	24	48
h	10	9	12	17
H_O	0.577	0.625	0.667	0.729
H_E	0.644	0.687	0.749	0.679
F_{IS}	0.106	0.092	0.112	−0.075
R	0.019	0.045	0.000	0.000
tps				
N	26	24	24	42
h	5	6	4	4
H_O	0.269	0.208	0.458	0.310
H_E	0.249	0.236	0.438	0.339
F_{IS}	−0.084	0.119	−0.048	0.089
R	0.000	0.000	0.000	0.026
hsp				
N	26	24	24	31
h	11	12	10	8
H_O	0.692	0.875	0.792	0.677
H_E	0.761	0.812	0.744	0.726
F_{IS}	0.092	−0.079	−0.066	0.067
R	0.013	0.025	0.000	0.000
rpl				
N	25	24	24	25
h	9	10	8	12
H_O	0.720	0.833	0.833	0.800
H_E	0.812	0.825	0.769	0.844
F_{IS}	0.116	−0.010	−0.086	0.053
R	0.027	0.000	0.000	0.012

Location abbreviations are defined in Table 1

N number of genotypes, h number of haplotypes, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient based on Weir and Cockerham (1984), R estimated null allele frequency

Table 7 Genetic diversity statistics for partial sequences of five nuclear loci from adults of *C. sapidus* collected in 2011

	RWR	FWC	LUM	GIL
ant				
N	16	24	24	21
h	8	15	11	8
H_O	0.375	0.417	0.625	0.476
H_E	0.810	0.834	0.790	0.769
F_{IS}	0.545*	0.506*	0.212	0.387
R	0.234	0.266	0.077	0.150
atps				
N	16	24	24	23
h	8	11	10	8
H_O	0.688	0.708	0.542	0.522
H_E	0.736	0.693	0.637	0.654
F_{IS}	0.068	−0.022	0.152	0.206
R	0.000	0.003	0.060	0.065
tps				
N	16	23	24	23
h	2	4	3	4
H_O	0.063	0.130	0.167	0.261
H_E	0.175	0.205	0.159	0.310
F_{IS}	0.651	0.368	−0.051	0.162
R	0.138	0.098	0.000	0.051
hsp				
N	16	24	24	23
h	10	9	9	9
H_O	0.813	0.792	0.917	0.696
H_E	0.806	0.743	0.768	0.795
F_{IS}	−0.008	−0.067	−0.199	0.128
R	0.042	0.000	0.000	0.014
rpl				
N	16	24	24	23
h	7	10	8	7
H_O	0.813	0.708	0.667	0.783
H_E	0.813	0.793	0.638	0.807
F_{IS}	0.000	0.108	−0.045	0.031
R	0.000	0.070	0.000	0.018

Location abbreviations are defined in Table 1

N number of genotypes, h number of haplotypes, H_O observed heterozygosity, H_E expected heterozygosity, F_{IS} inbreeding coefficient based on Weir and Cockerham (1984), R estimated null allele frequency

* Significant F_{IS} values after a standard Bonferroni correction ($p < 0.001$)

Differences between life stages

Megalopae were collected in 2010 from the four locations where adults were sampled in both 2010 and 2011 (Table 1). As was the case for adults, no significant heterogeneity in megalopal haplotype frequencies was found among samples from these locations (Table 8); therefore samples of megalopae were pooled for comparisons with pooled samples of adults. Megalopal haplotype frequencies were significantly different from those of adults collected in 2010 after a Bonferroni adjustment, with *atps* frequencies significantly different between the two groups (Table 8).

Table 8 Results of all exact tests of genetic differentiation with adult (A) and megalopae (M) *C. sapidus* from Louisiana

Year (life stage)	Locations	*p* value				All loci	
		atps	*tps*	*hsp*	*rpl*	χ^2_8	*p* value
2010 (A)	*All*[a]	0.0578	0.3451	0.0577	0.4108	15.3	0.0534
	RWR, *FWC*, *LUM*, *GIL*	0.1457	0.2944	0.0475*	0.5658	13.5	0.0948
2010 (M)	*RWR*, *FWC*, *LUM*, *GIL*	0.3005	0.1646	0.6542	0.5515	8.1	0.4284
2010 (A) vs. 2010 (M)	*All*[a,b] (A) versus *All*[b,c] (M)	0.0011**	0.7306	0.1063	0.4850	20.2	0.0097**
2011 (A)	*RWR*, *FWC*, *LUM*, *GIL*	0.3178	0.5538	0.6631	0.0114*	13.2	0.1036
2010 (A) vs. 2011 (A)	*RWR*	0.0594	1.0000	0.2928	0.1296	12.2	0.1430
	FWC	0.1537	0.1104	0.7312	0.1537	10.0	0.2677
	LUM	0.0183*	0.0114*	0.0306*	0.0456*	30.1	0.0002**
	GIL	0.4598	0.0222*	0.3983	0.1813	14.4	0.0713
	RWR, *FWC*, *LUM*, *GIL*[b]	0.0130*	0.4436	0.0499*	0.0037**	27.5	0.0006**
	RWR, *FWC*, *GIL*[b]	0.1636	0.5930	0.0604	0.1204	14.5	0.0693
2010 (M) vs. 2011 (A)	*RWR*	0.7056	1.0000	0.9444	0.0150*	9.2	0.3243
	FWC	0.1764	1.0000	0.0590	0.5970	10.2	0.2539
	LUM	0.3784	0.0540	0.1641	0.5099	12.7	0.1211
	GIL	0.3701	0.5577	0.0537	0.1143	13.3	0.1006
	RWR, *FWC*, *LUM*, *GIL*[b]	0.2055	0.5735	0.0501	0.0269*	17.5	0.0253*

* $p < 0.05$; ** $p < 0.01$ indicates significance at the appropriate Bonferroni-corrected α

[a] Includes all the Louisiana locations sampled in 2010: CAL, RBC, RWR, FWC, HBW, MIL, LUM, GIL, and LPT

[b] Pooled locations

[c] Includes all the Louisiana locations where megalopae were sampled: RWR, FWC, LUM, and GIL

Thus, megalopae and the adult population that presumably spawned them differed in *atps* haplotype frequencies. Megalopal haplotype frequencies were also significantly different from those of adults collected in 2011 (Table 8). However, in this case, it was haplotype frequencies at the *rpl* locus that were significantly different. Thus, megalopae differ in *rpl* haplotype frequencies from the adult population in which they would presumably be included, after having recruited to that population in the previous year. Separate comparisons between megalopae and the adults collected in 2011 for each of the four locations were not significant, although the comparison for the *rpl* locus at the RWR location would be significant without a Bonferroni correction (Table 8).

Differences between nGOM and distant locations

The AMOVA results for all loci, except *atps*, showed significant heterogeneity among samples from Louisiana, Texas, Mexico, and Venezuela (Table 9) after a standard Bonferroni correction ($\alpha = 0.0125$). Pairwise F_{ST} analyses were done in two ways: with the Louisiana adults pooled across years and with each year as a separate group. Because the results of both analyses were similar, only the pairwise F_{ST} and *p* values for the pooled samples are presented in Table 10. For all loci, the largest and only significant F_{ST}

Table 9 AMOVA results for each locus tested across the following samples: Louisiana 2010 adults, Louisiana 2011 adults, Louisiana megalopae, Texas, Mexico, and Venezuela

	F_{ST}	*p* value	Variation among populations (%)	Variation within populations (%)
atps	0.0031	0.1162	0.31	99.69
hsp	0.0242	0.0000*	2.42	97.58
rpl	0.0109	0.0005*	1.09	98.91
tps	0.1669	0.0000*	16.69	83.31

* Significant after a standard Bonferroni correction ($p < 0.0125$)

estimates were from comparisons between the sample from Venezuela and samples from either Louisiana or Texas (Table 10). This can be attributed to the haplotypes that occur at high frequencies in Venezuela (e.g., *atps_4*, *hsp_9*, *tps_5*, *rpl_2*) being absent or found at much lower frequencies at other locations (Supplementary Table 2).

Several haplotypes that were found in one or more of the samples collected outside of Louisiana were absent from the much larger sample from Louisiana (Supplementary Table 2), providing further evidence that sample sizes for Texas, Mexico, and Venezuela were adequate for broad comparisons. Nonetheless, given the exceptionally small sample size from Mexico (n = 5), the results from any

Table 10 Pairwise F_{ST} estimates and significance for all samples

	Megalopae	Adults	Texas	Venezuelaa	Mexico
atps					
Megalopae		0.3602	0.2397	0.0076	0.7161
Adults	0.0000		0.3052	0.0162	0.7358
Texas	0.0046	0.0016		0.3441	0.7536
Venezuela	0.0566	0.0440	−0.0006		0.3388
Mexico	−0.0292	−0.0335	−0.0468	0.0129	
hsp					
Megalopae		0.7483	0.6618	0.0000*	0.3587
Adults	−0.0015		0.8397	0.0000*	0.3822
Texas	−0.0069	−0.0096		0.0000*	0.8004
Venezuela	0.2400	0.2335	0.2311		0.1136
Mexico	0.0000	−0.0025	−0.0349	0.0720	
rpl					
Megalopae		0.6062	0.9789	0.0000*	0.4988
Adults	−0.0009		0.9233	0.0000*	0.4920
Texas	−0.0133	−0.0102		0.0001*	0.7218
Venezuela	0.1213	0.1138	0.1156		0.0844
Mexico	−0.0125	−0.0106	−0.0365	0.0852	
tps					
Megalopae		0.4788	0.5596	0.0000*	0.2274
Adults	−0.0010		0.4427	0.0000*	0.0632
Texas	−0.0077	−0.0040		0.0000*	0.4888
Venezuela	0.6774	0.7066	0.6741		0.0001*
Mexico	0.0280	0.0517	−0.0140	0.5924	

F_{ST} values are shown below the diagonal, and *p* values are shown above. Megalopae and adults were collected in Louisiana

* *p* values are significant after a standard Bonferroni correction ($p < 0.0013$)

comparisons that include this location should be interpreted with caution.

Discussion

Null alleles at *ant*

Evidence for null alleles at the *ant* locus consisted of significant heterozygote deficiencies at *ant,* but no other loci. Notably, one of the primers used for PCR amplification of *ant* matched an intron sequence; this was not the case for the other loci. This highlights another advantage of using protein-coding sequences as genetic markers over other markers amplified by PCR: primers can be designed to match relatively conserved exonic sequences, thus reducing the likelihood of null alleles. Heterozygote deficiencies can also result from non-random mating or Wahlund effects, but these would be expected to affect all loci. No null homozygotes were observed for *ant* (i.e., a product was successfully amplified from every individual). This suggests the occurrence of allele dropout, a phenomenon known to occur with microsatellites in which some alleles amplify more efficiently than others (Van Oosterhout et al. 2004). If this was the case, estimates of the frequency of the null allele would be unreliable. Null alleles are known to bias tests of population genetic differentiation (Chapuis and Estoup 2007), and we found that inclusion of *ant* substantially increased estimates of genetic differentiation observed across locations, years, and life stages (data not shown). For these reasons, we chose to exclude the *ant* locus from further analyses.

Spatial population genetic structure

The life history of blue crabs suggests that dispersal should be sufficient to prevent spatial and temporal heterogeneity in allele frequencies that might otherwise arise from barriers to gene flow (Steele 1991) (Costlow and Bookhout 1959). However, while homogeneity has been found in surveys of genetic markers for blue crabs, both slight and pronounced spatial and temporal heterogeneity have been reported as well (Berthelemy-Okazaki and Okazaki 1997; Kordos and Burton 1993; McMillen-Jackson et al. 1994). These unexpected findings raise the possibility that the exchange of larvae among blue crab populations in the nGOM could, at least in some situations, be severely limited by either barriers to dispersal or selection acting at early life stages. Either would have important implications for the management of blue crab fisheries, as well as raise questions of more general importance about the relationship between planktonic dispersal and gene flow.

Interpretation of patterns of variation at genetic markers is often dependent and constrained by the type of markers employed. For example, microsatellites are useful for detecting slight differences among populations, while mitochondrial DNA is better for resolving deep phylogeographic structure (see Bilodeau et al. 2005 for a nGOM example). In the present study, DNA sequences of protein-coding genes with known physiological roles were used because they combine the most desirable properties of allozymes, mitochondrial DNA, and microsatellites; they are derived from independently segregating diploid loci with known physiological functions, rich in polymorphism, objectively scorable, and not especially prone to homoplasy. In addition, we sampled across years, life stages, and at both relatively small (the coast of Louisiana) and large spatial scales (locations in Texas, Mexico, and Venezuela) to provide points of comparison for previous studies.

The findings of this study are mostly consistent with the expectation of high gene flow among populations of blue crabs in the nGOM. However, although nothing resembling the extreme heterogeneity over time, space, and life stages

reported by Kordos and Burton (1993) were observed, small differences were detected between the 2 years of the study and between samples of adults and megalopae. Differences in haplotype frequencies larger than those reported in any other blue crab study, with the exception of Kordos and Burton (1993), were also found in comparisons between nGOM samples and a sample from Venezuela. While genetic heterogeneity among the nine Louisiana locations sampled in 2010 was not significant at an α of 0.05, the nearly significant p value of 0.0534 does indicate that the differences in haplotype frequencies among samples cannot be readily explained by chance. Thus, it is very plausible that there is slight genetic heterogeneity among locations.

We found no evidence of pronounced genetic breaks for blue crabs at either the Chenier Plain or the Mississippi River. The location of a genetic break at the Chenier Plain for the burrowing ghost shrimp, *Callichirus islagrande* (Bilodeau et al. 2005), corresponds to a stretch of muddy habitat unsuitable for this species. In contrast, the Chenier Plain is not likely to be a barrier for blue crabs, which have a continuous distribution across the entire GOM. However, the zoea of blue crabs require salinities >20 ppt for growth (Costlow 1967), therefore the freshwater plume from the Mississippi River in the nGOM could reduce larval dispersal across the mouth of the river. Annual variation in the Loop Current and eddy activity are known to influence the retention and recruitment of blue crab larvae at locations east of the Mississippi River (Perry et al. 2003). In 2010, the repeated detachment and re-attachment of Eddy Franklin from the Loop Current, and the deep intrusion of the eddy into the nGOM, resulted in a westward flow (Hamilton et al. 2011), which may have facilitated larval dispersal and gene flow across the mouth of the Mississippi River during this year. Examining the spatial genetic variation in blue crabs across this potential boundary in additional years would be necessary to provide a more complete picture of connectivity between the eastern and western GOM.

Temporal population genetic structure

Large influxes of larvae from populations with extremely different genetic compositions has been suggested as a possible cause for marked temporal changes in allele frequencies observed in adult blue crabs in the nGOM (Kordos and Burton 1993; McMillen-Jackson and Bert 2004). Our survey, which includes a location within the range sampled by Kordos and Burton (1993) as well as a location to the south (MEX) and locations to the northeast (Louisiana), did not reveal any such highly divergent populations in the GOM, although one was found roughly 2,000 km distant in Venezuela. The temporal and life-stage differences that we found in our study were relatively small and thus would not require large influxes of larvae from very divergent populations to produce them. Furthermore, the temporal and life-stage differences we observed were differences in the frequencies of common haplotypes, and not the appearance of unusual haplotypes. Therefore, it is unlikely that these results were caused by influxes of larvae from an extremely divergent population like the one sampled in Venezuela.

Temporal changes in allele frequencies are sometimes attributed to strong genetic drift caused by sweepstakes reproduction, which can occur if genetically effective population sizes become very small (i.e., <1,000) as the result of extreme variance in reproductive success (Hedgecock 1994). Although blue crabs have the high fecundity required for sweepstakes reproduction, our findings are not consistent with its predicted effects. If a sweepstakes event had occurred in nGOM blue crabs, we would expect it to affect all locations, rather than just one. In addition, the combination of female migration to offshore spawning sites (Gelpi et al. 2009, 2013), a long larval period, and protracted spawning and recruitment seasons in the GOM (Daugherty 1952; Perry 1975; Perry and McIlwain 1986) would be expected to aggregate many recruitment events over multiple years into the adult population and thus dampen any transient signals of sweepstakes reproduction.

Another possible explanation for genetic differences over time or between life stages in benthic marine invertebrates is selection acting at post-recruitment life stages on genetic markers or loci linked to them. This was previously suggested to explain why megalopae collected at several locations along the Texas Coast were genetically differentiated from juveniles and adults collected nearby 6–12 months later (Kordos and Burton 1993). Although selection is a plausible explanation for these observations, more evidence would be needed to eliminate other explanations and make a compelling case for selection.

Deepwater Horizon Oil Spill

In 2010, during the time that we were sampling blue crabs in the nGOM, the Deepwater Horizon Oil Spill (DHOS) occurred off the coast of Louisiana, releasing oil continuously from April 20 through July 15, 2010, and nearly 7 million liters of the chemical dispersant Corexit® EC9500A were applied to accelerate the breakdown of the oil (Barron 2012). Oil was reported along more than 1,600 km of coastline in the nGOM during the summer of 2010, with the Louisiana shore and marshes experiencing some of the highest levels of oiling (Barron 2012). Mortalities and sublethal effects were also reported for marine organisms in the marshes and coastal waters of Louisiana (Barron 2012; Whitehead et al. 2011). Oil was seen at our sampling sites GIL, LPT, and on Marsh Island, Louisiana (near MIL), as well as within Terrebonne Bay, which is the entrance point

to LUM. The spill coincided with peak blue crab larval dispersal in offshore waters and, based on the trajectory of oil from the spill site, it is likely that blue crab larvae supplying the locations in this study would have come in contact with oil and/or dispersant. Both alone and in synergistic combination, oil and Corexit® are toxic to blue crabs (Wang and Stickle 1987; Fucik et al. 1995). In response to the oil spill, fishery closures in both state and federal waters resulted in approximately 24 % of the US Exclusive Economic Zone (EEZ) of the nGOM being closed to all fishing during the summer of 2010 (McCrea-Strub et al. 2011). These closures included shrimp trawling fisheries, which are known to impact blue crabs as bycatch. Thus, both positive and negative effects on blue crab populations could have resulted from the oil spill.

At present, we can only speculate about possible effects on the population genetic variables we measured in our study. Changes in larval mortality could have altered normal patterns of gene flow; changes in adult mortality could have altered the relative contributions of larvae from different source populations; and selection for resistance to oil or dispersant could have altered haplotype frequencies, particularly of genes involved in stress responses. Long-term studies are needed to establish normal spatial and temporal patterns of genetic variation in blue crabs against which extreme events such as the DHOS can be evaluated. A better understanding of blue crab biology in the nGOM is also needed, including linkages between specific spawning sites and sites of recruitment, the effects of offshore larval mortality on recruitment, and the potential for blue crabs to adapt to oil, dispersant, and other pollutants.

Conclusions

Previous studies presented an inconsistent picture of the extent to which genetic variation is structured in blue crab populations within the nGOM. If allowances are made for differences in statistical power, our study is consistent with those that found low but detectable levels of temporal and spatial heterogeneity. The findings of Kordos and Burton (1993) remain the exception. Our study paralleled theirs in using protein-coding loci (allozymes or gene sequences) as markers, in testing for temporal, spatial, and life-stage differences, and in including a sampling location within the range of their study. Despite these similarities, we did not observe the extreme heterogeneity they reported nor did we find evidence for the highly divergent populations within the nGOM that they hypothesized could act as exogenous sources of variation. However, we will continue to sample blue crabs along the Texas Coast, and we hope to extend the set of genetic markers we employ to include gene sequences of allozymes surveyed by Kordos and Burton (1993).

The present study introduces the use of DNA sequences for protein-coding genes from the nuclear genome for population genetic studies of blue crabs. As we have shown, these markers have many desirable characteristics, including sufficient polymorphism to detect small levels of population divergence (i.e., F_{ST} on the order of 0.01). They also have known physiological roles, which provides a more complete biological context in which to study the population genetic forces acting upon them. We encourage our colleagues to adopt the use of these markers for their own investigations, and to work with us in expanding the set of protein-coding loci included in studies of blue crabs.

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