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## Population structure of the Atlantic sand fiddler crab *Uca pugilator* along the eastern coast of US revealed by molecular data

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**Abstract** The Atlantic sand fiddler crab *Uca pugilator* is an extremely abundant crab found along the eastern coast of the United States. Fiddler crabs have a life cycle with an obligatory planktonic larval phase of 30–90 days, which might be expected to lead to widespread larval dispersal and consequent genetic homogeneity over considerable distances. However, a large amount of morphological and behavioral variation is found between northern and southern populations along the eastern coast. This study was undertaken to determine the population genetic structure of *U. pugilator* and to determine whether these differences may have a genetic basis. The population structure of the fiddler crab was analyzed using 472 individuals collected from 12 sites along the eastern coast. PCR-based single stand conformation polymorphism (SSCP) was used to investigate between-site variation in the mitochondrial 16S rRNA gene of these individuals. Analysis of genetic variation indicated frequent gene flow between nearby localities, but much reduced levels between populations separated by larger geographic distances. Thus, despite the potential for high dispersal by planktonic larvae, population differentiation and isolation by distance is evident between northern and southern populations of *U. pugilator*. A high amount of genetic differentiation ( $F_{ST} = 0.3468$ ) was found between northern and southern regions suggesting that the morphological and behavioral differences between these two regions have a genetic basis and may represent subspecies [*Current Zoology* 55 (2): 150–157, 2009].

**Key words** Atlantic sand fiddler crab, *Uca pugilator*, Decapoda, Molecular population genetics, Gene flow, SSCP, 16S rDNA

A continuing challenge in biology is to understand the processes by which populations become genetically distinct, especially in the marine environment. This genetic differentiation among local populations can provide indirect evidence of the pattern and scale of local dispersal (Avice, 2004). High dispersal potential is a common characteristic found in many marine organisms (Palumbi, 1996) and the genetic structure of most marine organisms often correlates with the level of dispersal of their larval stages (Kyle and Boulding, 2000). In general, species with larvae that spend a long time in the plankton afford opportunities for extensive gene flow that would reduce genetic differentiation across the geographic range (Palumbi, 1994; Avice, 2004). However, the expectation of reduced genetic differentiation in marine species with planktonic larvae fails to be met in some cases (Kordos and Burton, 1993). For instance, unperceived physical or biological barriers may interrupt gene flow despite a lengthy planktonic larval stage (Hedgecock, 1986).

Fiddler crabs (Decapoda: Ocypodidae, *Uca*) are a well-studied group of small, intertidal brachyuran crabs found in temperate salt marshes as well as subtropical and tropical intertidal communities throughout the world (Crane, 1975; Salmon and Kettler, 1987; Rosenberg,

2001). They are best characterized by strong sexual dimorphism and male asymmetry. Males have a single enlarged claw, used in ritualized contests and inter- or intrasexual signaling, that is paired with a small feeding cheliped, while females have paired small feeding chelipeds (Crane, 1975). Fiddler crabs have been the subject of numerous studies on sexual selection, reproductive isolation, visual and acoustic display, combat, foraging, asymmetry, allometry, regeneration, morphometrics, circadian rhythms, osmoregulation, heat tolerance, visual neurology, toxicity and environmental monitoring (reviewed in Rosenberg, 2001). Despite their use as a model system, there have been few attempts to employ molecular markers and molecular techniques to study fiddler crab biology. Molecular genetic techniques have been applied to the analysis of fiddler crab phylogeny (Levinton et al., 1996; Stumbauer et al., 1996; Kitaura et al., 1998), but studies on the population structure of the genus *Uca* are lacking (Hedgecock, 1986; Salmon and Kettler, 1987; Huang and Shih, 1995) and have typically been limited to allozyme studies.

The Atlantic sand fiddler crab *Uca pugilator* (Bosc, 1802) is found along the eastern coast of the United States in the low intertidal zones of both estuaries and exposed

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sandy coastal habitats from the northern shores of Cape Cod, Massachusetts to the southern coast of Florida (Crane, 1975). *U. pugilator* offers a unique opportunity to examine the influences of planktonic larvae on population structure and genetic diversity because of its long planktonic larval period and broad geographic range. Breeding in *U. pugilator* typically begins in early spring and continues throughout August (Colby and Fonseca, 1984; Morgan, 1996). Larval release occurs 13 days after mating (Christy, 1978) and occurs on the large amplitude, nocturnal spring high tides (Morgan, 1987; Morgan and Christy, 1995; Christy and Morgan, 1998) that favor larval emigration from estuaries (Morgan and Anastasia, 2008). Once released, larvae are rapidly flushed from the estuaries and may develop > 20 km offshore (Christy, 1982; Morgan, 1987; Epifanio, 1988; Epifanio and Garvine, 2001; Sanford et al., 2006; Morgan and Anastasia, 2008). Larvae then undergo a planktonic development of 6 – 8 weeks (Williams, 1984; Epifanio, 1988) before returning to suitable adult habitat along the banks of tidally influenced brackish and freshwater creeks (Brodie et al., 2005).

Notwithstanding the potential for some estuarine retention of larvae, the relatively long planktonic larval life of *U. pugilator* coupled with apparent export from estuaries leads to a prediction of high levels of dispersal among populations. To test this hypothesis, specimens were collected from localities spanning the eastern coast of the United States and haplotype data from the mitochondrial large ribosomal subunit (16S rRNA) gene was used to determine the extent of gene flow among

geographically separated populations of *Uca pugilator*. Determining the spatial scale at which gene flow occurs for *U. pugilator* is essential to our understanding of the population biology of and microevolution in this species and would contribute tremendously to our understanding of the biogeography and dispersal abilities of intertidal organisms.

## 1 Materials and methods

### 1.1 Sampling

*Uca pugilator* were collected at twelve localities along the Atlantic coast of North America during June and July 2006. Male *U. pugilator* were taken from 3 sites in Georgia, 3 sites in Florida, 2 sites in South Carolina, 1 site in New Jersey, 1 site in Virginia, and 2 sites in Massachusetts (Table 1; Fig.1). Immediately after collection, the large claw was obtained from male individuals by applying pressure at its articulation with the body until it was autotomized. The crabs were returned to the field. Use of claws as tissue sources reduced the impact on local populations. Males regenerate a claw that is removed. Also, the claw provides a large amount of tissue for extraction of DNA. A minimum of 40 claws was collected from each site. Claws were kept on ice or preserved in 100% ethanol until returned to the laboratory, where they were stored at  $-80^{\circ}\text{C}$ .

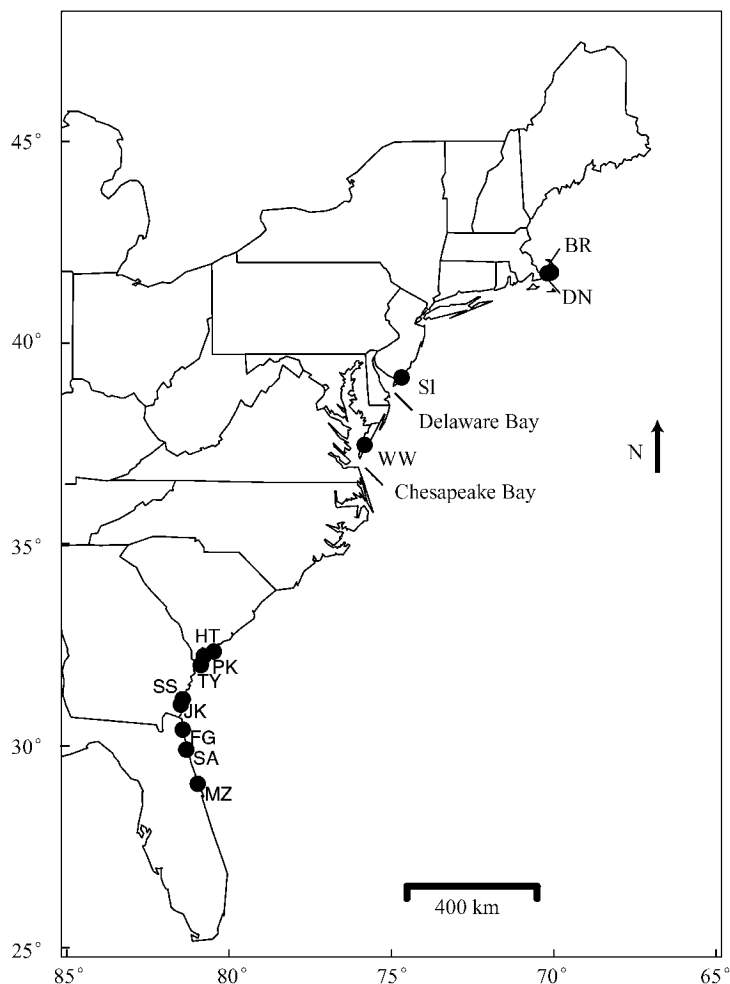
### 1.2 DNA extraction, primer design and amplification

Total genomic nucleic acid was extracted from the manus (containing the closer muscle) of each claw using cetyltrimethylammoniumbromide (CTAB) buffer extraction. In brief, ground samples were incubated at  $65^{\circ}\text{C}$  for

**Table 1** Sampling locations, dates, number of samples ( $n$ ), haplotype frequencies, and haplotype diversity of *Uca pugilator* from the Atlantic coast

| Collection site | Collection date | $n$ | Haplotypes |       |       |       |       | Haplotype diversity ( $h$ ) |
|-----------------|-----------------|-----|------------|-------|-------|-------|-------|-----------------------------|
|                 |                 |     | A          | B     | C     | D     | E     |                             |
| North           |                 | 162 | 0.370      | 0.574 | 0.025 | 0.031 | 0.000 | $0.5350 \pm 0.0230$         |
| BR              | 7/31/2005       | 37  | 0.162      | 0.757 | 0.027 | 0.054 | 0.000 | $0.4084 \pm 0.0906$         |
| DN              | 7/29/2005       | 44  | 0.318      | 0.568 | 0.045 | 0.068 | 0.000 | $0.5825 \pm 0.0545$         |
| SI              | 7/25/2005       | 36  | 0.389      | 0.611 | 0.000 | 0.000 | 0.000 | $0.4889 \pm 0.0408$         |
| WW              | 7/23/2005       | 45  | 0.577      | 0.400 | 0.022 | 0.000 | 0.000 | $0.5172 \pm 0.0366$         |
| South           |                 | 310 | 0.539      | 0.048 | 0.019 | 0.000 | 0.003 | $0.2868 \pm 0.0300$         |
| PK              | 7/6/2005        | 41  | 0.731      | 0.269 | 0.000 | 0.000 | 0.000 | $0.4024 \pm 0.0656$         |
| HT              | 7/6/2005        | 33  | 0.727      | 0.273 | 0.000 | 0.000 | 0.000 | $0.4091 \pm 0.0726$         |
| TY              | 6/23/2005       | 47  | 0.787      | 0.213 | 0.000 | 0.000 | 0.000 | $0.3423 \pm 0.0693$         |
| SS              | 7/7/2005        | 34  | 0.852      | 0.118 | 0.029 | 0.000 | 0.000 | $0.2656 \pm 0.0921$         |
| JK              | 6/19/2005       | 39  | 0.821      | 0.103 | 0.051 | 0.000 | 0.026 | $0.3212 \pm 0.0919$         |
| FG              | 7/8/2005        | 40  | 0.850      | 0.100 | 0.050 | 0.000 | 0.000 | $0.2718 \pm 0.0869$         |
| MZ              | 7/8/2005        | 35  | 0.886      | 0.086 | 0.029 | 0.000 | 0.000 | $0.2134 \pm 0.0879$         |
| SA              | 7/8/2005        | 41  | 1.00       | 0.000 | 0.000 | 0.000 | 0.000 | $0.0000 \pm 0.0000$         |
| All sites       |                 | 472 | 0.674      | 0.292 | 0.021 | 0.011 | 0.002 | $0.4610 \pm 0.0183$         |

See Fig.1 for a description of site abbreviations.



**Fig. 1** Sampling localities of *Uca pugnator* along the East Coast of North America

BR = Brewster, MA (41°45.42'N; -70°04.58'W); DN = Dennis, MA (41°43.45'N; -70°12.07'W); SI = Sea Isle City, NJ (39°09.09'N; -74°41.52'W); WW = Willis Wharf, VA (37°28.35'N; -75°51.46'W); HT = Hunting Island, SC (32°21.05'N; -80°27.55'W); PK = Pinckney Island, SC (32°14.07'N; -80°46.86'W); TY = Tybee Island, GA (32°00.75'N; -80°52.16'W); JK = Jekyll Island, GA (31°01.84'N; -81°25.10'W); SS = St. Simons Island, GA (31°10.23'N; -81°25.35'W); FG Ft. George Island, FL (30°24.51'N; -81°25.91'W); SA = St. Augustine, FL (29°54.62'N; -81°18.80'W); MZ = Matanzas River, FL (29°45.85'N; -81°15.68'W).

20 minutes in 400  $\mu$ L of CTAB buffer (50 mmol/L Tris-HCl pH 8.0, 4 mol/L NaCl, 1.8% CTAB, and 25 mmol/L EDTA pH 8.0), 250  $\mu$ L of lysis buffer (4.5 mol/L guanidine thiocyanate, 25 mmol/L sodium citrate, 0.5% lauryl sarcosine), and 50  $\mu$ g of proteinase K. Next, nucleic acids were separated from the cellular debris in chloroform. Nucleic acids were precipitated in alcohol. Pelleted nucleic acids were washed with 70% and then with 100% ethanol. Finally, the nucleic acids were resuspended in 10 mmol/L Tris-HCl. Detailed methods are described in Mixson et al. (2004).

A 307 bp segment of the 16S rDNA gene was targeted using specifically designed primers. Primer design was based on a conserved region deduced from the review of eight DNA sequences, four from *U. pugnax* (Genbank Accession #s Z79672-Z79675) and four from *U. pugnator* (Genbank Accession #s Z79659-Z79662) (Stumbauer et al., 1996). The primer sequences used

were: Upg16S\_79F, 5' GRA ATC TTG TAT GAA TGG YTG AAC and Upg16S\_386RC, 5' TAA CGC TGT TAT CCC TYA AGT AA. PCR reactions, in 25  $\mu$ L volumes, were carried out in a Perkin-Elmer 9600 thermal cycler. The PCR reaction consisted of 1  $\times$  PCR buffer, 2.5 mmol/L MgCl<sub>2</sub>, 200  $\mu$ mol/L of each dNTP, 2.5 U of hot-start *Taq* DNA polymerase, 200  $\mu$ mol/L of each primer, and 0.5  $\mu$ L (0.1 – 0.5  $\mu$ g) of DNA template. The samples were amplified under the following conditions: an initial denaturation at 94°C for 15 minutes for hot-start polymerase followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 56.8°C for 90 seconds, and extension at 72°C for 90 seconds. After 36 cycles there was a final extension at 72°C for 10 minutes.

### 1.3 Single-strand conformation polymorphism

Positive PCR products were subjected to single strand conformation polymorphism (SSCP) using a Bio-Rad Protean II xi Cell (Bio-Rad, Hercules, Ca). SSCP

gels were cast with 12.0 ml of acrylamide stock solution (38:1 acrylamide:bis-acrylamide), 6.0 ml of  $5 \times$  TBE, 3.0 ml of glycerol, and 39 ml of distilled water. Samples were prepared by adding 5.0  $\mu$ l of SSCP loading buffer (98% formamide (v/v), 0.05% bromophenol blue (w/v), and 0.05% xylene cyanole blue (w/v)) to 2.5  $\mu$ g of PCR product with 100  $\mu$ mol/L of forward and reverse primers and 1  $\mu$ l of 15% ficoll (w/v). The samples were denatured at 98°C for 9 minutes followed by snap cooling on ice for 5 minutes to promote folding of the single stranded DNA. Samples were then loaded onto the gel. A total of four gels were run in two Bio-Rad Protean II xi cells simultaneously at 60 mA for 12–14 hours at 16°C. Each gel was stained with ethidium bromide for 30 minutes, viewed under UV light, and photographed to provide a record. Single strand conformation polymorphism gels were scored by eye. Samples with identical banding patterns were assumed to have identical sequences and were assigned the same haplotype/genotype. Shifts in band mobility indicated different secondary structures and were assigned to different haplotypes/genotypes (Dowling et al., 1996). To insure accuracy, a sample of recognized haplotypes from previous gels was included on later gels to facilitate comparison and haplotype assignment.

#### 1.4 Statistical analysis

The number of mtDNA haplotypes and haplotype diversities ( $h$ ) (Nei, 1987) were calculated for each collection site and geographic region using the program ARLEQUIN 3.1 (Excoffier et al., 2005).  $G$ -tests were used to assess differences in the haplotype frequencies between collection sites and between geographic regions using the computer program JMP version 6.0. (Sall et al., 2001). To determine the amount of genetic differentiation between collection sites pairwise  $F_{ST}$  comparisons were calculated using the ARLEQUIN program. Collection sites were grouped into geographic regions (North and South) based on geographic proximity and whether they were north or south of a natural physiographic feature, the Chesapeake Bay. An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was performed with the ARLEQUIN program to test for variation within and among regions. Relationships between the 12 collection sites were visualized through a UPGMA dendrogram based on Nei's unbiased minimum distances (Nei, 1978) (Fig.2). Lastly, Mantel tests (Mantel, 1967), implemented in the program TFPGA (Tools For Population Genetic Analysis; Miller, 1997) with 10000 permutations, were performed to test for correlations between geographic distances and pairwise  $F_{ST}$  values.

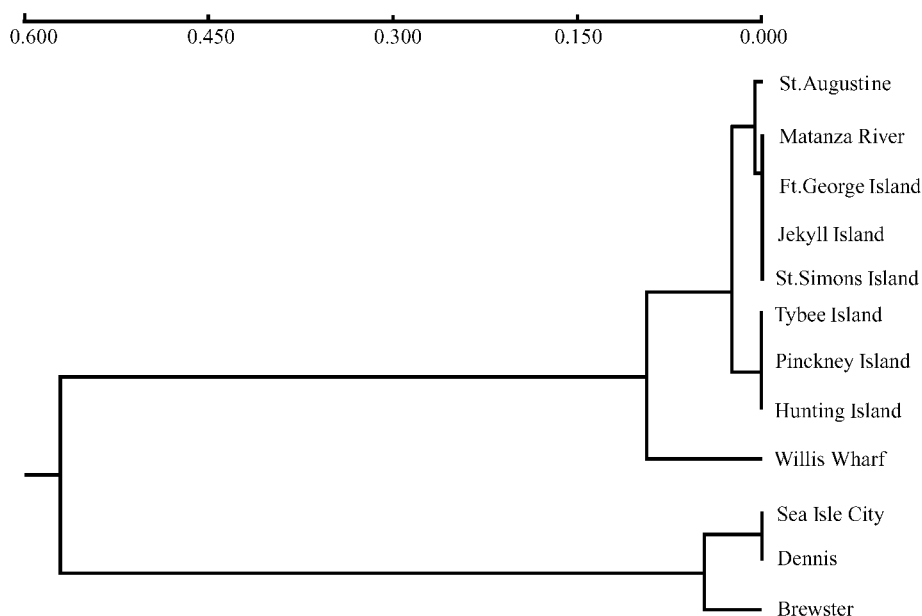


Fig.2 UPGMA dendrogram showing the genetic relationships among 12 collection sites of *Uca pugilator* based on Nei's unbiased genetic distance

## 2 Results

### 2.1 Haplotype sampling and distribution

High molecular weight DNA was extracted from 514 of 576 crabs collected from 12 sites (Fig.1). Four hundred and eighty-three samples were successfully

amplified with 472 being successfully scored with SSCP. Five distinct haplotypes of *U. pugilator* were identified (Table 1). In general, there was greater haplotype diversity in the northern than southern sites. Only one site, St. Augustine, was fixed for a particular haplotype. Haplotype A was the most frequent haplotype, occurring

in 67% of the samples. Haplotype A was the only widespread haplotype, being shared among all populations. In contrast, haplotype D was unique to the two Massachusetts locations while haplotype E was only found at the Jekyll Island location. Haplotype A was the majority haplotype (> 83%) at the southern locations and a minority haplotype (< 37%) at the northern locations.

## 2.2 Genetic diversity and population structure

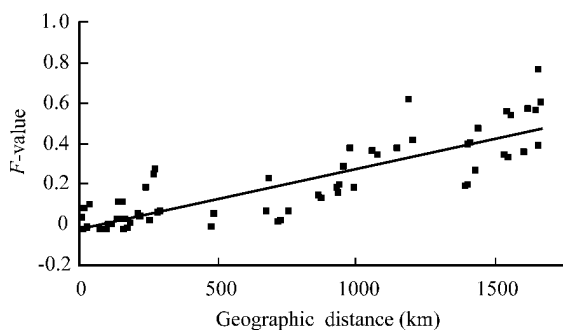
Haplotype diversity was high for the northern collection sites ( $h > 0.5350$ ), but was much lower and ranged between 0.0000 (St. Augustine) and 0.4091

(Hunting Island) for the southern sites (Table 1). Significant genetic differentiation was observed between many of the collection sites as shown by both the pairwise  $F_{ST}$  comparisons (Table 2) and the analysis of haplotype frequency variation among sites ( $G = 173.747$ ,  $P = 0.000$ ). Allele frequencies also varied significantly between North and South regions ( $G = 111.397$ ,  $P < 0.0001$ ) while allele frequencies among sites within regions showed no significant variation. There was a strong correlation between geographic and genetic distance across all populations (Mantel test,  $r^2 = 0.6448$ ,  $P < 0.001$ , Fig.3).

**Table 2** Pairwise  $F_{ST}$  (lower triangle) values as a measure of genetic differentiation for *Uca pugilator* collection sites and geographic distance (km) (upper triangle) between sites

|    | BR     | DN     | SI     | WW     | PK     | HT     | TY     | SS     | JK     | FG     | SA     | MZ   |
|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|------|
| BR | –      | 10     | 487    | 687    | 1415   | 1406   | 1444   | 1548   | 1560   | 1620   | 1659   | 1670 |
| DN | 0.034  | –      | 477    | 678    | 1405   | 1396   | 1434   | 1538   | 1550   | 1610   | 1650   | 1661 |
| SI | 0.055  | –0.013 | –      | 212    | 939    | 930    | 957    | 1061   | 1077   | 1149   | 1192   | 1205 |
| WW | 0.230* | 0.065  | 0.052  | –      | 729    | 720    | 759    | 864    | 876    | 938    | 981    | 993  |
| PK | 0.402* | 0.198* | 0.193* | 0.021  | –      | 9      | 30     | 135    | 147    | 216    | 268    | 284  |
| HT | 0.395* | 0.187* | 0.183* | 0.015  | –0.028 | –      | 29     | 144    | 166    | 224    | 275    | 291  |
| TY | 0.475* | 0.266* | 0.286* | 0.066* | –0.015 | –0.016 | –      | 104    | 116    | 186    | 239    | 255  |
| SS | 0.561* | 0.346* | 0.365* | 0.141* | 0.028  | 0.030  | –0.002 | –      | 12     | 86     | 143    | 161  |
| JK | 0.538* | 0.330* | 0.347* | 0.131* | 0.027  | 0.028  | 0.001  | –0.024 | –      | 98     | 155    | 173  |
| FG | 0.569* | 0.359* | 0.379* | 0.154* | 0.040  | 0.042  | 0.008  | –0.027 | –0.023 | –      | 39     | 77   |
| SA | 0.766* | 0.564* | 0.617* | 0.376* | 0.250* | 0.276* | 0.183* | 0.111* | 0.109* | 0.096* | –      | 19   |
| MZ | 0.605* | 0.391* | 0.416* | 0.184* | 0.061  | 0.065  | 0.021  | –0.025 | –0.017 | –0.023 | 0.076* | –    |

\*  $P < 0.05$ . See Fig.1 for a description of site abbreviations.



**Fig.3** Relationship of  $F_{ST}$  values and geographic distance of collection sites for *Uca pugilator*

$r^2 = 0.6448$ ;  $P < 0.001$ .

Collection sites were grouped into two regions based on geographic proximity. The North region consisted of the Willis Wharf, Virginia site (WW in Fig.1) and all sites farther north while the southern region consisted of the Hunting Island, South Carolina site (HT in Fig.1) and all sites farther south. Significant variation in haplotype frequencies occurred between the regions ( $G = 111.397$ ,  $P < 0.000$ ). The haplotype diversity in the North ( $h = 0.5350$ ) was higher than in the South (0.2868) (Table 1). The differentiation between regions

( $F_{ST}$ ) was 0.3468 (genetic distance = 0.3866).

Most collection-site pairwise comparisons failed to reveal genetic differentiation between sites within a region (Table 2). Exceptions occurred for the St. Augustine, Florida site in the South region (Table 2). All collection sites in the North region were genetically differentiated from those in the South with the exception of the Willis Wharf, Virginia site which did not differ from South Carolina sites (Table 2). The hierarchical AMOVA analyses comparing the North and South regions revealed a large variance in genetic structure between the regions (FCT = 0.341,  $P < 0.0001$ , Table 3). Only a small (3.5%), yet significant, amount of the total variance was ascribed to variation among sites within regions ( $F_{sc} = 0.053$ ,  $P < 0.0001$ ).

## 3 Discussion

This study implements the use of molecular markers to infer the dispersal capabilities of *Uca pugilator* and to investigate the genetic population structure of this crab along the Atlantic coast. High levels of gene flow resulting in genetic homogeneity of geographically separated populations were predicted for *U. pugilator* due to the long duration of free swimming planktotrophic

**Table 3 Analysis of molecular variance (AMOVA) of the 16S rDNA fragment for two different regional groupings**

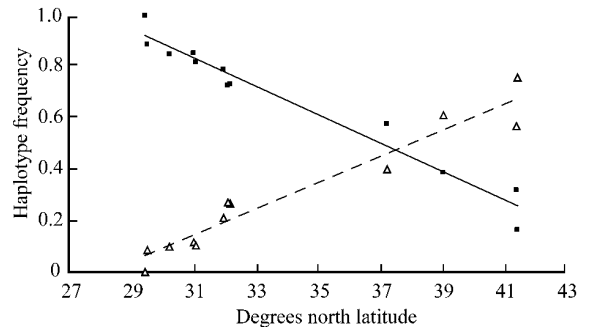
| Source of variation        | df  | Variance component | % total variation | F-statistics   | P-value |
|----------------------------|-----|--------------------|-------------------|----------------|---------|
| Among regions              | 1   | 0.097 $V_a$        | 34.05             | 0.341 $F_{CT}$ | 0.000   |
| Among sites within regions | 10  | 0.010 $V_b$        | 3.51              | 0.053 $F_{SC}$ | 0.000   |
| Among sites                | 460 | 0.178 $V_c$        | 62.44             | 0.376 $F_{ST}$ | 0.000   |

zoaeae. However, the significant association between genetic and geographic distance, based on variation of 16S rDNA, suggests restricted levels of gene flow over large distances and indicates a case of isolation by distance. Thus, a higher degree of genetic differentiation between populations is observed than would be predicted for a species possessing a widely-dispersing planktonic larval stage. The level of genetic structuring across the range of *U. pugilator* clearly demonstrates that restricted gene flow can occur in organisms with long-term planktonic larva with an apparently high dispersal potential. The paradigm that organisms with pelagic larvae generally exhibit high levels of gene flow and low levels of genetic differentiation is not supported by this study.

As with fiddler crabs, planktonic larval development is the most common reproductive strategy among tropical and subtropical marine species (Epifanio et al., 1988). Thus, high dispersal potential characterizes many marine organisms (Palumbi, 1996) and, if realized, could result in genetic cohesion among geographically isolated populations. However, the extent to which high frequency and/or long-distance dispersal of planktonic larvae impact the population genetics of marine species is unclear (Kordos and Burton, 1993). Reduced dispersal may result from generally unperceived physical or biological barriers to movement even when there is a lengthy planktonic larval stage (Hedgecock, 1986). For instance, tolerance of pelagic larvae to physical conditions such as temperature and salinity may limit dispersal in fiddler crabs (Sanford et al., 2006).

Clinal patterns in allele frequencies are common in many marine organisms with high dispersal potential and are often attributed to local selection pressures or local retention of larvae due to oceanographic forces and larval behavior (Koehn, 1969; McMillen-Jackson and Bert, 2004). These clines are common in marine species and are often attributed to selection due to differential tolerances to local environmental temperatures, salinity, or other factors (McMillen-Jackson et al., 1994). For the Atlantic sand fiddler crab haplotype frequencies showed regional geographic structuring in the form of a distinct latitudinal cline throughout the species range and is evident when haplotype frequencies are plotted against latitude (Fig. 4). There appears to be a moderate amount of gene flow occurring between local populations but

reduced levels are occurring between geographic regions. Koehn (1969) demonstrated a similar cline in allozyme frequencies in the blue crab, *Callinectes sapidus*, along the Atlantic coast and attributed it to temperature-dependent selection regimes. This distribution of haplotypes seen in *U. pugilator* may be a consequence of similar local selection pressures and development of this cline may then be facilitated by factors limiting dispersal and promoting isolation between distant populations (McMillen-Jackson et al., 1994).

**Fig. 4 Geographic variation in allele frequency**

Latitude of collection sites plotted against frequency of the two most common alleles. Black squares represent frequency of haplotype A ( $r^2 = 0.9516$ ;  $P < 0.001$ ) and open triangles represent frequency of haplotype B ( $r^2 = 0.9285$ ;  $P < 0.001$ ).

The spatial scale at which populations of *U. pugilator* are isolated is probably determined by river outflows, ocean currents, and features of the coastline that may shield some regions from the influences of currents. It appears from our study that natural populations, delimited by reasonable expectations of gene flow, may span hundreds of kilometers of coastline. For instance, the Brewster, Massachusetts site differs significantly from the Willis Wharf, Virginia site, nearly 700 km away, but not from the Sea Isle City, New Jersey site, 500 km away. This provides evidence that the Delaware Bay, but not the Chesapeake Bay (Fig. 1), may act as a major barrier to dispersal for *U. pugilator*. The Delaware Bay has been shown to act as a barrier to dispersal for other marine organisms as well (Duvernell et al., 2008). Similarly, most southern sites exhibit no significant differentiation across nearly 300 km (Hunting Island, South Carolina to Matanzas River, Florida) providing evidence that local gene flow is occurring. The St. Augustine site is unusual in differing from other southern sites. Perhaps, the bay there does not receive larvae from outside because of barrier islands and a natural bight in the coast result in currents missing the area. Sites that are over 750 km apart differ significantly with respect to pairwise  $F_{ST}$  values. This plus the above observations suggests geographic structuring on a scale of 300–500 km despite the appearance of higher levels of gene flow on a local scale. Gene flow between populations

a few hundred kilometers apart may account for the high rates diversity within sites.

Morphological, behavioral, and physiological variation across the range of *U. pugilator* suggests local adaptation and corroborates our genetic evidence of reduced long distance gene flow. For instance, in some southern populations the carapace of these crabs is pink rather than the typical purplish color (Crane, 1975). Also, body size increases from north to south (see Miller, 1973; Colby and Fonseca, 1984; Yoder et al., 2007). In northern, but not southern populations, subfreezing temperatures are tolerated, with hibernation occurring when cold temperatures are encountered (Crane, 1975; Demeusy, 1975). Behavior also differs between northern and southern populations. Males in northern populations do not build hoods (sediment domes) over entrances to breeding burrows as do males in southern populations (Crane, 1975). Moreover, northern and southern populations differ in how the claw is waved (Crane, 1975), the frequency of waving, and the frequency and intensity of ritualized contests (personal observation).

From Florida to Virginia, crabs form feeding droves on sand flats that may contain thousands of individuals. Across the same region, males at burrows wave throughout much of the day during and near new and full moons, and males frequently engage in contests, using the claw, to obtain or maintain control of a breeding burrow. In contrast, in New Jersey and Massachusetts, crabs do not drive, wave only infrequently, and rarely engage in contests. Thus, the change in behavior is relatively abrupt.

It is clear that there is significant differentiation between northern and southern parts of the range of *Uca pugilator*. This differentiation occurs over a distance of just over 1000 km and is associated with variation in morphology and social behavior. The genetic differentiation seen in *U. pugilator* indicates that moderate population structure occurs along the Atlantic coast in the form of a regional latitudinal cline resulting from high local gene flow and limited gene flow over larger geographic distances throughout the species range. The limited gene flow between geographic regions suggests that morphological and behavioral differences across the geographic range may have a genetic basis. Cryptic speciation in marine environments is a phenomenon that is now recognized in many groups of organisms and has been suggested for many crustaceans (Hedgecock, 1986; Knowlton, 1986, 1993). This study documents genetic differentiation between *U. pugilator* populations and highlights the need to evaluate the population structure of this species further to determine if in fact the division of *U. pugilator* into subspecies would be justified as suggested by Crane (1975).

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