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## Molecular Population Genetics of the Atlantic Sand Fiddler Crab, *Uca Pugilator*, Along the Atlantic Coast

David A. Weese

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MOLECULAR POPULATION GENETICS OF THE ATLANTIC SAND FIDDLER  
CRAB, *UCA PUGILATOR*, ALONG THE ATLANTIC COAST

by

DAVID A. WEESE

(Under the Direction of Quentin Fang and Denson K. McLain)

ABSTRACT

The Atlantic sand fiddler crab, *Uca pugilator*, is an extremely abundant fiddler crab found along the eastern and Gulf 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 576 individuals collected from 12 sites along the eastern coast. PCR-base single stand conformation polymorphism (SSCP) was used to analyze segments of the mitochondrial 16S rRNA and the nuclear internal transcribed spacer 1 (ITS1) genes of these individuals. The ITS1 marker did not prove to be informative when screened by SSCP for this study. The 16S marker revealed a moderate amount of population structure ( $F_{ST} = 0.292$ ) between populations. The results of this study reveal frequent gene flow between nearby localities, but reduced levels between populations separated by large distances. Despite the potential for high dispersal by planktonic larvae, population differentiation and isolation by distance was found

between populations *U. pugilator*. Northern and southern regions are separated by a genetic distance of 0.3866 suggesting the potential for morphological and behavioral differentiation across the species range.

INDEX WORDS: *Uca pugilator*, Fiddler crab, Population structure, Gene flow, Larval dispersal, Single-stand conformational polymorphism, 16S rDNA, ITS-1

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## CHAPTER 1

### INTRODUCTION

#### *Uca pugilator*

Fiddler crabs (Ocypodidae, *Uca*) are a well-known group of small, intertidal brachyuran crabs (Rosenberg, 2001). The Atlantic sand fiddler crab, *Uca pugilator* (Bosc 1802), is extremely abundant along the eastern and Gulf coast of the United States. Its range extends from the northern shores of Cape Cod, Massachusetts to Key West, Florida and along the Gulf Coast of Florida (Crane, 1975). Sand fiddler crabs are typically found in great abundance along sheltered shores, *Spartina* marshes, and along tidal creeks with sandy or muddy sand substrate. Other species that can be found throughout *U.*

*pugilator's* range include the Atlantic marsh fiddler crab, *Uca pugnax*, and the red-jointed fiddler crab, *Uca minax*, in the northern more temperate range and the mudflat fiddler crab, *U. rapax*, across the southern range (Crane, 1975).

*U. pugilator* can be easily distinguished from these sympatric species due to its unique coloration and several morphological characteristics of the claw. The carapace of these crabs has a distinctive purplish violet coloration that easily distinguishes them from *U. pugnax* or *U. minax*. In the more southern populations the carapace is sometimes pink rather than the purplish color (Crane, 1975). These are relatively small fiddler crabs with the size of the crabs varying with latitude, increasing from north to south in carapace width (Colby and Fonseca, 1984, Pratt and McLain, unpublished). In the northern range, *U. pugilator* is smaller and adapted to subfreezing temperatures, hibernating regularly where cold temperatures are encountered (Crane, 1975). Colder temperatures negatively

affect growth in two ways: (1) by inhibiting molting (Miller and Vernberg, 1968) and (2) by limiting feeding (Colby and Fonseca, 1984).

Like all *Uca* species, males of *U. pugilator* possess one enlarged cheliped that is used in a variety of functions mainly related to mating. To attract mates, male crabs stand outside their burrows waving their cheliped. Males use a lateral-circular wave, but in the reverse direction characteristic of other species (Crane, 1975). The waving pattern of male crabs varies across the specie's range. In the most southern populations the cheliped makes a single jerk midway to its highest reach, but in northern populations this jerking motion is absent (Crane, 1975). Once a male attracts a female, the mating occurs in mating burrows. In the southern populations, these burrows can be distinguished from temporary-burrows by the presence of a semi-circular sediment hooded-dome over the entrance (Christy, 1982) constructed with the major cheliped. The northern populations lack these domed breeding burrows and are relatively inactive socially (Crane, 1975). *U. pugilator* also differs in several behavior aspects through its range. In the more southern parts of the species range, droving is highly developed and is lacking in the northern populations (Crane, 1975). Along the species northern boundary the crabs appear to be relatively inactive socially (Crane, 1975). Despite these difference in display, behavior, and physiology and the vast geographic range of these crabs they are considered a single species. The genetic background is lacking for this species and any present attempt at subdivision is unjustified.

Breeding in *U. pugilator* typically begins in early spring (March) and continues throughout August (Morgan, 1996; Colby and Fonseca, 1984). Females may select males either by the waving display or the burrow size. Once a female has chosen her mate she

enters the burrow preceded by the male. After oviposition occurs, the male seals the female in a chamber and returns to the surface to continue to court females. Females emerge days later and release planktonic larvae into the estuarine water 13 days after mating (Christy, 1978). Tides occur twice a day along the east coast, but vary in amplitude during the breeding season with the larger tidal amplitudes occurring around full and new moons. The reproductive behavior of female crabs and the timing of larval release correlate with these spring high tides. Females release eggs typically on large amplitude, nocturnal ebb tides (Christy and Morgan, 1998; Morgan, 1987; Morgan and Christy, 1995). Larva then undergoes planktonic larval development in the ocean and return as adults to the banks of tidally influenced brackish and freshwater creeks (Brodie *et al.* 2005). The planktonic period for a typical brachyuran crab is usually between thirty and ninety days (Epifanio, 1988).

Planktonic larval development is the most common reproductive strategy among tropical and subtropical marine species (Epifanio, 1988). The adaptive significance of the timing of larval release by crabs has been well studied. Morgan and Christy (1995) suggest that the timing of larval release to coincide with the high spring tide is in response to selection pressure of predators. The synchronous hatching of larvae may facilitate the swamping of predators. Another advantage of the reproductive cycle of fiddler crabs is to overcome habitat patchiness. The relation of larval release and the tide cycles may be an adaptation to increase the probability that the final stage of the larva will be transported by tidal currents to substrates suitable for adults (Christy, 1978). Planktonic larval development may also aid in high dispersal rates and high levels of gene flow among dispersed populations. Presumably, the outgoing tide assures

movement of larvae from the parental estuary (Epifanio, 1988). Planktonic larval development is common among marine animals and has several advantages which include protection against predators, potential to take advantage of newly available habitat, increase gene flow, and to overcome habitat patchiness.

The genetic differentiation among local populations can provide indirect evidence of the pattern and scale of local dispersal (Avice, 1994). The genetic structure of marine animals often correlates with the level of dispersal of their larval stages (Kyle and Boulding, 2000). Most marine species with high dispersal potential show limited population differentiation, because dispersal ability and high gene flow are often positively correlated (Hansen, 1980). Marine organism with larvae that are in the plankton for long periods of time should show little to no genetic differentiation over long distances. There are only a few known examples where high levels of population genetic structure have been found in marine invertebrate species with planktonic larvae (Kyle and Boulding, 2000). In these exceptions, reasons for the unexpected high levels of genetic structure include behavioral mechanisms that limit dispersal (Palumbi, 1994), oceanographic currents (Wares *et al.*, 2001), and historic barriers to gene flow (Avice, 1992). The ability to assess the degree of genetic differentiation between populations allows one to infer the dispersal abilities of the species in question.

The Atlantic sand fiddler crab has been intensively studied in the past. Crane (1975), Christy (1983), Salmon and Hyatt (1983), and many others (reviewed in Rosenberg 2001) have contributed greatly to our knowledge of the biology of the sand fiddler crab. Despite the great number of studies on the sand fiddler crab there have been few studies to employ molecular markers and molecular techniques to study the biology

of the fiddler crab. Molecular genetic techniques have been applied to the analysis of fiddler crabs phylogeny (Levinton *et al.*, 1996; Sturmbauer *et al.*, 1996; Kitaura *et al.*, 1998) but not to the analyses of the population structure of the crab. In general, studies on the population structure of the genus *Uca* are rare (Salmon and Kettler, 1987; Hedgecock *et al.*, 1982; and Huang and Shih, 1995). Genetic analysis of the *U. pugilator* could provide valuable insight on the amount of gene flow that may occur between populations and would contribute tremendously to our understanding of the biogeography and dispersal abilities of this crab.

### **Population Genetics**

Population genetics is the study of genetic variation within and between species that attempts to understand the processes resulting in evolutionary change through time (Hartl and Clark, 1997). Genetic variation within species is important because it makes up the driving force of evolution. Population genetics attempts to explain the origin of genetic variation and patterns, explain the organization of genetic variation, and to understand the mechanisms that cause changes in allele frequencies (Conner and Hartl, 2004). The patterns of genetic variation that exists within a species can lead to the understanding of the evolutionary history and evolutionary processes of the species studied. These patterns of genetic variation in an organism can be investigated by studying the population structure of the species.

Species always exhibit some extent of geographical structure which results in a nonrandom pattern in the spatial distribution of alleles (Hartl, 2000). A single species is not a panmictic group of individuals, but is subdivided into a set of local populations. Local populations are the fundamental units of population biology (Hartl and Clark,



1997). Patterns of spatial genetic variation within a species are a result of population subdivision. Genetic variation between populations can occur when favorable habitats are isolated, which is known as environmental patchiness (Hartl, 2000). Environmental patchiness disrupts the gene flow between localities. Low levels of gene flow result in an increase in genetic variation among populations population structure. The spatial dynamics of genetic variation influences many different evolutionary processes (Skalski, 2004).

Many studies of geographical structure of marine populations have been conducted along the Atlantic coast (Weinberg *et al.*, 2003). Population subdivision is common in many marine species with or without life histories that support high dispersal rates (Palumbi 1994). Many factors can restrict gene flow and lead to population structure in marine species, such as geographic barriers, the influence of ocean currents, natural selection, behavioral limits to dispersal, and historically barriers to migration (Schizas *et al.*, 1999, Lavery *et al.*, 1996, and Avise, 1992). How long-distance dispersal of planktonic larva affects the population structure of marine species remains controversial (Kordos and Burton, 1993). It could be predicted that a species with high frequency and/or long-distance dispersal capabilities of planktonic larva could overcome geographic isolation and barriers to gene flow.

*U. pugilator* is a species appropriate for population genetic studies because of its broad geographic range, but studies on population genetic structure of the genus *Uca* are rare (Huang and Shih, 1995). The few studies of population genetic structure of fiddler crabs are contradictory. Studies by Salmon and Kettler (1987) and Hedgecock *et al.* (1982) imply that the level of genetic variation and genetic differentiation within and

among *Uca* species are low. However, an isozyme study of *Uca arcuata* in Taiwan (Huang and Shih, 1995) showed a high level of genetic variation within populations and moderate levels between populations of *U. arcuata*. Clearly, further work on this group is necessary. Here, I investigate the population structure of *U. pugilator* using modern molecular techniques.

### **Molecular Techniques**

Molecular ecology entails discovering the spatial and temporal scales over which genetic structure occurs. Evolutionary biologists are now equipped with a variety of recently developed, high-resolution genetic markers which allows them unprecedented capacity to portray genetic structure of natural populations (Grosberg *et al.*, 1996). The development of allozyme markers in the 1960's opened the door to genetic characterization of population structures. For the first time, numerous individuals could be analyzed at relatively low cost and with few technical problems. Over the last two decades the rapid development of new molecular tools that provide access to highly variable DNA markers has made high-resolution analysis of genetic structure possible (Avice, 1994). Most of these methods are either time-consuming or expensive, and sometimes both. Most involve complex protocols and expensive equipment and require large amounts of undegraded DNA or destructive sampling. As long as cost is an object, time a constraint, or technical ability limiting, molecular ecologist will face a trade-off between gathering high-resolution genetic information and sampling large numbers of individuals (Lessa and Applebaum, 1993). Thus, sequencing samples of DNA is still the limiting step in many projects (Sunnucks *et al.*, 2000). With large sample sizes, sequencing every individual becomes largely impractical. Polymerase chain reaction-

single-strand conformation polymorphisms (PCR-SSCP) provides a rapid, inexpensive, and highly sensitive way to analyze variation in DNA samples (Dean and Milligan, 1998; Girman, 1996; Hayashi, 1992; Sunnucks, 2000).

Developed in the late 1980s (Orita *et al.*, 1989), SSCP offers a very sensitive method of detecting single base pair changes in DNA samples (Hayashi, 1992). Today, SSCP is one of the most widely used techniques for the analysis of DNA. A target sequence of DNA is amplified with specific primers using a PCR reaction. The PCR products are then denatured resulting in single-stranded DNA, which is subjected to electrophoresis in a non-denaturing polyacrylamide gel. The principle of this technique is that the mobility of the nucleic acid is dependent on mass and conformation. When the double-stranded DNA is denatured, some strands will fold upon themselves and adopt a conformation determined by intra molecular interactions dependent on sequence composition. Any change in the base sequence of the DNA will change the conformation of the molecule, which will result in different electrophoretic mobility. Even a one base pair difference in the sequence of the DNA can cause a change in the secondary structure of the molecule, leading to a difference in mobility. Thus, SSCP is the simplest and most sensitive method for detection of mutations (Hayahi, 1992).

PCR-SSCP is perhaps one of the most sensitive of the PCR-based techniques for detection of mutations (Orita *et al.*, 1989; Hayashi, 1992). SSCP is most sensitive for fragments about 100 base pairs in length (Girman, 1996) and reveal single base pair changes 99% of the time (Lessa and Applebaum, 1993). For fragments of around 200 base pairs, the detection rate is more than 90% (Hayashi and Yandell, 1993), and greater than 80% for fragment of 300-400 base pairs (Hayashi and Yandell, 1993; Girman, 1996;

Hayashi, 1991). Overall, SSCP can typically detect over 90% of sequence variation in most regions within the size range commonly used in these studies (Sunnucks *et al.*, 2000). The high sensitivity of PCR-SSCP makes it very useful in population genetics studies.

PCR based SSCP has other advantages. SSCP enables the rapid screening of a large number of samples (Girman, 1996), which is a necessity for a good population structure study. The extreme sensitivity of SSCP is also invaluable for these types of studies since a large portion of genetic variation is due to a single base difference (Sheffield *et al.*, 1993). Also, after the PCR products have been run on a SSCP gel they can be recovered for direct sequencing and analysis. There are some problems with the use of SSCP. The technique requires extensive optimization. Another disadvantage is the limited size of the DNA that can, 350-400 base pairs (Sunnucks *et al.*, 2000; Hayashi and Yandell, 1993; Girman, 1996; Hayashi 1992). Some researchers circumvent this problem by dividing long sequences into shorter fragments before the SSCP analysis (Hayashi 1992).

### **Research Objectives**

The Atlantic Sand Fiddler Crab has been intensely studied in the past, but few studies have attempted to examine the genetic population structure of these crabs. Based on its biology, *Uca pugilator* is predicted to express little genetic structure along the Atlantic coast. Despite this prediction, *U. pugilator* displays a high level of morphological and behavioral variation throughout its range. The objective of this study is to investigate the population structure of *Uca pugilator* along the Atlantic coast using molecular techniques. More specifically, the goals of this study are to (1) investigate the

degree of genetic variation within and among populations of *Uca pugilator*; (2) determine if northern and southern populations of *U. pugilator* vary genetically; and (3) evaluate the effectiveness of the 16S rDNA and ITS-1 sequences in studying the population structure of fiddler crabs.

## CHAPTER 2

### MATERIALS AND METHODS

#### Sample Collection

Samples of *Uca pugilator* were collected from several different localities along the Atlantic coast. A total of 12 sites were sampled along the east coast of the United States. *U. pugilator* samples were collected 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). These sites covered the entire range of the species with the exception of southern Florida. At each collection site the major cheliped was removed from 40-50 male crabs. Crabs were returned to the field. Samples were kept on ice or preserved in 100% ethanol until returned to the laboratory, where they were frozen at -80°C for storage.

#### DNA Extraction

For most samples, nucleic acid was extracted from the carpus and the merus (depending on the size) of each claw using a Masterpure DNA purification kit (Epicentre, Madison, WI.) following the manufacture's protocols. Samples were ground using a pestle in a 1.5 ml microcentrifuge tube with 300µl of lysis buffer and 1µl of proteinase K to lyse the cells and degrade any proteins. The samples were incubated at 65°C for 10 minutes and vortexed after 5 minutes. 150µl of protein precipitation reagent was added to the lysed sample which was then pelleted by centrifugation for 10 minutes at 8,000 G to separate out the cellular debris from the DNA. The supernatant was then transferred to a new microcentrifuge tube and 500µl of isopropanol was added. The tubes were then inverted 30-40 times to precipitate the DNA after which the samples were centrifuged for

10 minutes at 13,600 G. The supernatant was poured off and the DNA pellet was washed in 1ml of 75% ethanol. Samples were then dried in a DNA speed vac and resuspended in 70µl of 10 mM Tris-HCl.

For the remaining samples, nucleic acid was extracted following a CTAB extraction protocol. Samples were ground with a clean pestle in a microcentrifuge tube containing 400µl of cetyltrimethylammoniumbromide buffer (CTAB) [50mM Tris-HCL pH 8, 4 M NaCl, 1.8% CTAB, 25mM EDTA pH 8], 250 µl of lysis buffer, and 1µl of proteinase K. The samples were then incubated at 65°C for 20 minutes, and vortexed every five minutes. Five hundred microliters of chloroform: isoamyl alcohol (24:1) was then added to the samples. The samples were then mixed and centrifuged for 5 minutes at 15,800 G. The supernatant was then transferred to a second microcentrifuge tube and 500µl of chloroform was added. The samples were mixed and centrifuged for 5 minutes at 15,800 G. The supernatant fluid was transferred to a third microcentrifuge tube and a volume of 3M sodium acetate equal to one tenth of the volume the supernatant fluid was added. A volume equal to both the supernatant fluid and sodium acetate of 100% cold isopropanol was added next. Samples were then placed on ice for 10 minutes. To precipitate the DNA, the samples were then centrifuged at 4°C for 12 minutes at 15,800 G. The supernatant was discarded and the pellet was washed with 1 ml of cold 70% ethanol and centrifuged for 3 minutes at 15,800 G. The ethanol was poured off and the pellet was washed with 100% ethanol. The samples were centrifuged for another 3 minutes after which the ethanol was poured off and the pellet was dried for 1 minute in the DNA Speed Vac Drier. The pellet was resuspended in 50µl of 10mM Tris-HCL.

To verify the presence of high molecular weight DNA all samples were subjected to electrophoresis on 1.0% agarose gel at 70 volts for one hour. Following extraction, all samples were stored at -20°C for later use.

### **Primer Design**

Primers were developed to amplify the 16S rDNA region of the mitochondrial genome and the nuclear rDNA internal transcribed spacer 1 (ITS1). For the 16S rDNA region a total of 8 DNA sequences were downloaded from Genbank. These included four DNA sequences from the species *U. pugnax* (Accession #s Z79672-Z79675) and four DNA sequences from *U. pugilator* (Accession #s Z79659-Z9662) (Sturmbauer, *et al.*, 1996). The primers flanking the 16S rDNA region were as follows: (1)Upg 16S 79F, 5' GRA ATC TTG TAT GAA TGG YTG AAC and (2) Upg 16S 386R, 5'TAA CGC TGT TAT CCC TYA AGT AA. These primers target a 307 base pair portion of the 16S rDNA gene. For the forward ITS1 primers, the following 18S rDNA sequences were aligned and compared: *Dorsophila melanogaster* (Accession # M21017) (Tautz *et al.*, 1998), *Aedes punctator* (CPU22136) (Miller *et al.*, 1997), *Ocyopode quadrata* (AY743942) (Babbitt and Patel, unpublished), and *Ixodes kopsteini* (L76352) (Black *et al.*, 1997). Primers flanking the ITS1 region were Upg ITS1970F, 5'-CACACCGCCCGTCGCT ACTA and (2) Upg ITS1R1, 5'-GACCCATGAGCCGAGTGAT. The ITS primers target a region of about 400 base pairs of the internal transcribed spacer 1 between the 5.8S and 18s ribosomal subunits.

All sequences were aligned using the BioEdit Sequence Alignment Editor computer package (Hall, 1999). Primers were constructed from the most conserved regions of the alignment and checked for GC content, primer length, annealing



temperature similarity and the possibilities of secondary structures such as primer dimers and hairpin structures using Net Primer (PREMIER Biosoft International, 2005).

### **Polymerase Chain Reaction**

PCR amplifications were preformed using a Perkin-Elmer 9600 thermal cycler in 25µl volumes. The PCR mix consisted of Qiagen 10X PCR buffer, 2.5mM MgCl<sub>2</sub>, 200 µM of each dNTP, 2.5U of hot-start Taq DNA polymerase, 0.2µM of each primer, and 0.5µl (0.1-0.5µg) 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.

To avoid DNA contamination, the PCR reaction mixture was prepared under a sterile hood that was only used for PCR. All pipetters, tubes, pipette tips, and other equipment were treated regularly with UV light. To confirm DNA amplification, 5µl of PCR product was loaded onto a 1% agarose gel with 5µl of loading buffer and electrophoresed at 70V. All gels were run with a 1 kb standard DNA marker and both positive and negative controls.

### **Single Strand Conformation Polymorphism (SSCP)**

Positive PCR products were then subjected to single strand conformation polymorphism (SSCP) using a Bio-Rad Protean II xi Cell (Bio-Rad, Hercules, Ca). SSCP gels were cast by mixing 12.0 ml of acrylamide stock solution (38:1 acrylamide:bis-acrylamide), 6.0ml of 5X TBE, 3.0ml of glycerol, and 39ml of distilled water. The solution was degassed for 10-15 minutes then mixed with 100µl of 25%

ammonium persulfate and 100 $\mu$ l of TEMED to polymerize the gel. The gel was then pipetted between two glass plates. The gel was left to polymerize for 4-5 hours or overnight.

For the 16S rDNA, samples were prepared by adding 5.0 $\mu$ l of SSCP loading buffer (98% formamide (v/v), .05% bromophenol blue (w/v), and .05% xylene cyanole blue .05% (w/v)) to 2.5 $\mu$ g of PCR product with 100 $\mu$ M of forward and reverse primers and 1 $\mu$ l of 15% ficoll (w/v). The samples were denatured at 98 $^{\circ}$ C for 9 minutes followed by snap cooling on ice for 15 minutes to promote folding of the single stranded DNA. The 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 $^{\circ}$ C. Each gel was stained with ethidium bromide for 30 minutes and viewed under UV light and photographed for record keeping.

For the ITS-1, samples were prepared by adding 5.0 $\mu$ l of SSCP loading buffer (98% formamide (v/v), .05% bromophenol blue (w/v), and .05% xylene cyanole blue (w/v)) to 1.5 $\mu$ g of PCR product with 100 $\mu$ M of forward and reverse primers. The samples were denatured at 98 $^{\circ}$ C for 9 minutes followed by snap cooling on ice for 30 minutes to promote folding of the single stranded DNA. The 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 12-15 hours at 23 $^{\circ}$ C. Each gel was stained with ethidium bromide for 30 minutes and viewed under UV light and photographed for record keeping.

SSCP 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

different haplotype/genotypes. To insure accuracy, a sample of known haplotype from previously run gels was included in the group to use for comparison.

### **Statistical Analysis**

Haplotype frequencies were calculated for the 16S rDNA for all collection sites. A G test was used to assess differences in the haplotype frequencies between collection sites (JMP version 6.0.0 2005 SAS Institute Inc., Cary, NC). A p-value of less than 0.05 indicated that haplotype frequencies differed significantly between sites. To determine populations, a pairwise  $F_{ST}$  (Wright 1931, 1943, 1951) comparison was performed using Arlequin 3.1 (Excoffier *et al.*, 2006), a software program that analyzes population genetics data. Collection sites were grouped into populations if the  $F_{ST}$  comparison showed no significant difference between sites. G tests were used to assess differences in the haplotype frequencies between regions and among collection sites within regions. The haplotype diversity index (Nei, 1987) was calculated using Arlequin to assess the amount of genetic diversity for each site and population. The Arlequin program was used to run an analysis of molecular variance (AMOVA) and population  $F_{ST}$  comparisons. The AMOVA analysis (Weir and Cockerham, 1984; Excoffier *et al.*, 1992; Weir, 1996) calculated  $F$  statistics to partition diversity among populations and among regions. Where  $F_{CT}$  is the variance among populations relative to the total variance,  $F_{SC}$  is the variance among sites within populations, and  $F_{ST}$  is the variance among sites relative to the total variance. Nei's unbiased genetic identity and unbiased genetic distance (Nei, 1978) were calculated using Tools For Population Genetic Analysis (TFPGA) version 1.3 (Miller, 1997). Genetic identity values range from 0.0 to 1.0 depending on how similar or different haplotype frequencies are between populations. Genetic distance is the inverse

of genetic identity and is a measure of how unrelated two populations are from one another. MEGA version 3.1 (Kumar, Tamura, and Nei, 2004) was used to construct a neighbor joining tree using the genetic distances. To determine isolation by distance, the correlation between collection pairwise  $F_{ST}$  values and geographical distances were analyzed. Significance of the correlation was determined using a Mantel test (Mantel, 1967; Smouse *et al.*, 1986). Alleles In Space (Miller, 1997) was used for the mantel test using 100 replicates.

## CHAPTER 3

### RESULTS

A total of 576 *U. pugilator* crabs were collected from twelve sites along the Atlantic coast (Table 1). Of these, high molecular weight DNA was extracted from 514 samples (Fig. 2). Four hundred and eighty-three samples were successfully amplified with primers for the 16S rRNA gene (Fig. 3) and 502 sample were successfully amplified with internal transcribed spacer 1 (ITS1) primers (Fig 4). A total of 472 samples were successfully analyzed and scored with SSCP for the 16S rRNA locus (Fig. 5), with 5 haplotypes recognized (Table 2). Scoring of 373 samples was attempted for the ITS1 region, but the sample could not be scored with confidence.

#### **Haplotype Frequencies**

For the 16S rRNA locus, 5 haplotypes are identified in SSCP genotyping and labeled A to E. Haplotype A is the most common haplotype occurring in 67% (Table 3) of the individuals. The second most frequent haplotype is B (29%). These haplotypes (A and B) occurred at all sites except the St. Augustine site. The remaining haplotypes (C-E) are rare occurring infrequently among sites. South-to-north clinal variation occurs in the pattern of frequency distributions of haplotypes when haplotype frequencies are plotted against collection site (Fig. 6). To determine the amount of genetic diversity for each population the haplotype diversity was calculated for each population (Table 5). The northern populations possess more variation than that of the southern populations. The haplotype diversities vary between sites and regions and range from 0.0000 at the St. Augustine, FL site to 0.5825 at the Dennis, MA site. Significant variation in haplotype frequencies occurs between all sites and between all regions (Table 4). Allele

frequencies vary significantly between all sites ( $G=173.747$ ,  $p<0.0000$ ) and between all populations ( $G=147.896$ ,  $p<0.0000$ ). Allele frequencies among sites within populations show no significant variation.

### **Genetic Structure of *U. pugilator* populations**

To determine the genetic structure of *U. pugilator*, sites with pairwise  $F_{ST}$  values that were not significantly different were grouped into populations and populations were divided by significant pairwise  $F_{ST}$  values (Table 6). The 12 sampled sites represent 4 populations. Population 1 consists of two sites: Brewster and Dennis, Ma. Population 2 contains 3 sampled sites: Sea Isle City, NJ, Pinckney Island, SC, and Hunting Island, SC. Population 3 consists of five populations: Tybee Island and St. Simon's Island, Georgia and Ft. George Island and the Matanza River sites in Florida. The fourth population consists of only the St. Augustine, FL site. The haplotype diversities for the populations 1, 2, 3, and 4 are 0.5065, 0.4498, 0.2853, and 0.000 respectively (Table 5). Once grouped into populations, all pairwise comparisons of  $F_{ST}$  estimates are significant between all populations (Table 7).

Results from the AMOVA (Table 8) indicate that most genetic variation occurs within populations, accounting for 70.22% of the variation at the 16S rRNA locus (Table 8;  $F_{ST}= 0.29781$ ). Twenty nine percent of the variation is due to differentiation among populations (Table 8;  $F_{CT}= .29169$ ). Only a small portion, 0.61%, of the variation is due to differentiation among sites within populations (Table 8;  $F_{SC} = 0.00864$ ). The F-statistic values are significant for all sources of variation (Table 8).

A strong correlation between  $F_{ST}$  values and geographic distance between sites (Table 6) is found for the 16SrRNA locus ( $r = 0.8030$ ,  $p < 0.012$ ). Over 65% of variation

is explained by the geographic distances between sites (Fig. 7). Values for Nei's genetic unbiased identity and Nei's unbiased genetic distance (Nei, 1978) were calculated for between all populations (Table 9). Nei's unbiased genetic identities range from 0.4128 to 0.9883 between populations. Genetic distances range from 0.0411 to 0.8849 between populations.

All collection sites were divided into north and south geographic regions based on geographic location. The north region consists of both Massachusetts sites and the New Jersey and Virginia sites. The south region consists of all remaining collection sites. Nei's genetic distance and identity between the regions are 0.3866 and 0.6794 respectively. Results from the regional AMOVA (Table 8) indicate that 34% of the total genetic variation can be explained by the variation between the two regions (Table 8;  $F_{CT} = 0.34050$ ,  $p < 0.002$ ), while 62% of the variation occurs within the collection sites of the two regions (Table 8;  $F_{ST} = 0.37565$ ,  $p < 0.000$ ).

A neighbor-joining tree representing the genetic distance between populations and was created using Nei's unbiased genetic distance (Fig. 8). There is little difference between populations 3 and 4. These two populations are separated by a branch length of 0.091. These population, are only separated geographically by 62km and consist of all Georgia and Florida Sites. Populations 3 and 4 are clustered together and separated from population 2, the New Jersey and South Carolina sites, by a genetic distance of 0.268 and an average geographical distance of 950km. Populations 1, 2, and 3 are all separated from population 4 by a genetic distance of 0.415.

## CHAPTER 4

### DISCUSSION

This study implements the use of molecular markers to indirectly measure the dispersal capabilities of *Uca pugilator* and to investigate the genetic population structure of this crab along the Atlantic coast. The genetic structure and significant association between genetic and geographic distance, based on 16S rRNA, suggest that *U. pugilator* sites along the Atlantic coast experience restricted levels of gene flow over large distances. Consequently, 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 for *U. pugilator* found in this study clearly demonstrates restricted gene flow can occur in organisms with planktonic larva, in spite of 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. This study 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).

#### **Larval Dispersal**

The fact that haplotypes A and B are found at all collection sites, with the exception of the St. Augustine site (where only A occurs), may suggest that some level of gene flow is occurring between all populations and indicates some dispersal capability of *U. pugilator* larvae. Haplotype sharing can be a direct result of contemporary gene flow (Hurwood *et al.* 2003). Despite the evidence of haplotype sharing, haplotypes are not evenly distributed throughout the populations sampled. G tests showed significant



variation in the frequencies of 16S haplotypes between all sites and between all populations. A clinal variation occurs in haplotype distribution from south to north. The observed clinal distribution of haplotypes suggests that the dispersal capabilities of this crab are limited to small geographic scales. The higher haplotype diversities found in northern populations compared to southern populations suggest that genes may be flowing from northern populations southward. The lack of significant variation in the haplotype frequencies among sites within populations supports the fact that gene flow occurs regularly on a local scale. The distribution of haplotype frequencies and G test results suggest that gene flow frequently occurs between neighboring populations creating a stepping-stone effect between all populations.

The conclusion that *Uca pugilator* demonstrates restricted gene flow over limited geographic areas, in spite of high dispersal potentials is further supported by the genetic population structure of *U. pugilator* populations. Pairwise  $F_{ST}$  values were calculated between all collection sites. An  $F_{ST}$  value measures how genetically different are two populations. An  $F_{ST}$  value of 0 means that two populations are genetically identical and would have extensive amounts of gene flow occurring between the two populations. An  $F_{ST}$  value of 1, on the other hand, would mean that the two populations are genetically distinct from one another with no gene flow occurring between them. Collection sites with  $F_{ST}$  values that are not significantly different from one another are combined into one population. Collection sites that are grouped together into populations are geographically close to one another, suggesting that gene flow is occurring over short distances. Once grouped into populations, a pairwise comparison of population  $F_{ST}$  values reveals that there is restricted gene flow between populations. Pairwise  $F_{ST}$

comparisons of collection sites and populations reveal that the dispersal of *U. pugilator* larvae occurs over distances of 200-700km, but is negligible over distances over 1,000km.

The analysis of molecular variance of *U. pugilator* populations reveals a significant proportion of the observed genetic variation occurs within individual collection sites (~70%;  $F_{ST} = 0.298$ ;  $P < 0.001$ ) and between populations (~30%;  $F_{CT} = 0.292$ ;  $P < 0.001$ ). This data also suggests that gene flow is restricted between populations. The lack of variation among collection sites within populations demonstrates that gene flow occurs freely between collection sites within a population, but may be restricted to a local scale. Because the dispersal capabilities of adult fiddler crabs are negligible, the high amount of variation between populations can be attributed to limitations in larval dispersal over long distances.

Genetic differentiation between *U. pugilator* populations along the Atlantic coast results from a general pattern of restricted gene flow and isolation by distance. Thus, there are evidently limits to the distances these crabs can disperse. The four *U. pugilator* populations are highly structured with restricted gene flow between populations and even more so between northern and southern regions. Population 1, which consists of crabs collected from Massachusetts and Virginia, is separated from all other populations by the highest genetic distance (0.4146), suggesting a high level of divergence from the more southern populations (populations 2, 3, and 4). Populations 3 and 4, consisting of the Florida and Georgia collection sites, are more closely related to each other and are geographically closer to each other than to any other populations. Crabs collected from population 2 are separated from Georgia and Florida populations by a smaller genetic

distance than from the northern most population. This suggests that more gene flow occurs within geographically local populations, but is reduced over long distances.

The degree of genetic differentiation among local populations of marine species provides important indirect evidence, of the scale of effective local dispersal (Kim *et al.* 2003). Most marine organisms with high dispersal capabilities express little population structure, because gene flow usually correlates with dispersal capabilities (Hansen, 1980). Population genetic studies have revealed mild levels of population structure over regional scales in some marine species with high dispersal potential (Plaumbi, 1994). These studies suggest that gene flow occurs between populations, but there are often limits to the actual dispersal of these marine species.

Two hypotheses concerning the adaptive significance of larval release cycles in *Uca* spp. exist in which larvae are either exported or retained within estuaries (Morgan, 1987). Early investigations of the Atlantic coast have shown that fiddler crab larvae are not retained in estuaries, but rather the timing of larval release promotes seaward transport of larvae on ebb tides where they develop in the mouth of estuaries or over the adjacent continental shelf (Christy, 1982; Christy and Morgan, 1998; Epifanio *et al.*, 1998; Morgan, 1987). It has been suggested that this rapid seaward dispersal of crab larvae may be the first leg of a true migration (Strathmann, 1982) that increases larval survival by avoiding high predation rates, starvation, or physiological stress (Christy, 1982; Christy and Morgan, 1998; Morgan, 1987). Christy and Morgan (1998) state that crab larvae are often abundant 10 kilometers from shore indicating extensive dispersal may be common in this crab. This hypothesis is not support by the results of this study.

Low levels of gene flow were found between populations separated by small geographic distances suggesting *U. pugilator* lacks extensive dispersal capabilities.

There is evidence that despite being transported to the mouth of primary estuaries, crab larvae are retained in the estuary and return to recolonize their habitats (Epifanio, 1988). Early stage larvae are flushed from the marsh environment soon after hatching (Christy, 1982), but late-stage larvae take advantage of subtidal landward flow to actively remain in the primary estuary (Dittel and Epifanio 1982). Christy (1978) concluded that this was an adaptive behavior to overcome habitat patchiness: female *U. pugilator* release larvae at a time such that larvae experience maximum up-estuary transport when they are ready to colonize suitable habitat. Based on this hypothesis, it would be predicted that each estuary would be genetically distinct, because larvae would return to recolonize adult habitats and would not disperse to adjacent estuaries. This study does not support this prediction. There is evidence of restricted gene flow between populations, but each collection site was not genetically distinct. This suggests that gene flow may be restricted to a local scale and that there is evidence of larval dispersal over small geographic distances.

The genetic structure of *U. pugilator* populations and low levels of dispersal implied by this study supports the hypothesis that *Uca* spp. larvae are routinely flushed from tidal creeks and develop in the primary estuary and on the adjacent continental shelf (Lambert and Epifanio, 1982). Epifanio *et al.* (1988) found that there is some retention of all larval stages within the estuary, but that some larvae are carried to the adjacent continental shelf. The genetic structure of *U. pugilator* populations described in this study is congruent with the idea of retention and dispersal of larvae from primary

estuaries. The high genetic distances between populations can be explained by the few number of larvae that are carried past the continental shelf and are transported over long distances. *U. pugilator* exhibits much variation in the period of planktonic existence of the larval stage, 30-90 days (Epifanio, 1988). The possibility of a long larval period makes it possible that once larvae escape the tidal currents of the estuary, they could disperse long distances before settling on suitable habitat. The higher levels of gene flow between populations separated by relatively short distances is due to the fact that the larvae that develop on the continental shelf may be transported to other estuaries, while those developing in the estuaries take advantage of tidal currents to promote their transport upstream (Epifanio, 1988). The apparent southward flow of genes between populations is due to southward surface currents right along the Middle Atlantic Bight coast (Pape and Garvine 1982) and the southward surface current dominating the outer shelf (Bumpus 1965).

Genetic differentiation between *U. pugilator* populations along the Atlantic coast results from a general pattern of restricted gene flow and isolation by distance. There are evidently limits to the distances these crabs can disperse as larvae. Fiddler crabs may have the potential to disperse through ocean currents, but this occurs infrequently, at least over long distances. This finding is consistent with an emerging pattern indicating that some marine organisms, even those known to migrate long distances to reproduce or with the potential for widespread larval dispersal, can retain significant population structure (Romand and Palumbi, 2004).

## Regional Variation

One of the objectives of this study was to assess levels of genetic differentiation between northern and southern fiddler crabs. This was done partly to establish whether morphological and behavioral differences might be under genetic or environmental control. Haplotypes D and E are unique haplotypes found in the north and south regions respectively. The presence of unique haplotypes in each region implies that gene flow between regions is restricted. In the north region haplotype B is the dominant haplotype found at all collection sites. In the south region haplotype A is the most common haplotype and is fixed at the St. Augustine site. The significant variation in haplotype frequencies found in each region also indicates a low level of gene flow between the two regions. If gene flow regularly occurs between the two regions haplotypes should be distributed evenly between the two areas. The uneven distribution of haplotypes is the first indirect evidence of restricted gene flow between the two regions.

Nei's genetic distance between the two regions studied is 0.3866. This value is higher than what has been reported for the genetic population structure within and between other *Uca* species (Hedgecock *et al.*, 1982; Huang and Shih, 1995; Salmon and Kittler, 1987) and is comparable to the genetic distance found between 7 species of Malaysian fiddler crabs (Suzawa *et al.*, 1993). High levels of genetic differentiation between populations are usually caused by restricted levels of gene flow or genetic drift within populations (Wright, 1978). The  $F_{ST}$  value between these two regions is relatively high,  $F_{ST}=0.3468$ . The high  $F_{ST}$  value found between regions indicates a strong break in gene flow between the two areas. Extremely high  $F_{ST}$  values (eg.  $>0.5$ ) would generally indicate zero gene flow (Hurwood *et al.*, 2003).

From the results of this work, it is clear that between the northern and southern range of *Uca pugilator*, a distance of a little over 1,000km, there is significant differentiation between the two regions. The high amount of genetic variation found between these two regions correlates with the large amount of variation seen in the morphology, acoustic behavior, and social behavior seen in these crabs. This differentiation between the regions indicates that there is likely to be little or no gene flow between the two areas and that the above mentioned differences may have a genetic basis. These results suggest that *U. pugilator* may be comprised of two sibling species: a southern species and one northern species that varies from its southern counterpart morphologically, behaviorally, and genetically. The evidence from this study supports Crane's (1975) claim that the division of the species *Uca pugilator* into species or subspecies may be desirable. Cryptic speciation in marine environments is a phenomenon that is now recognized in many groups of organisms and has been suggested for many crustaceans (see a review in Knowlton, 1986 and 1993). Regional variation in behavior, acoustic behavior, and morphology is already known. This study provides an insight into the amount of genetic variation that occurs between regions. The level of differentiation between the two regions found in this study highlights the fact that more in-depth genetic analyses of these crabs are warranted.

### **ITS-1 As A Genetic Marker**

Congruence among multiple data sets is arguably the most reliable indicator of phylogenetic accuracy (Willows-Munro *et al.* 2005). Current population genetic studies often utilize a multi-marker approach; most often using both mitochondrial and nuclear makers. Using both nuclear and mitochondrial markers may yield different evolutionary

histories. This study attempted to apply both a mitochondrial marker (16S rRNA) and a nuclear marker (ITS-1). The 16S marker revealed a small amount of variation in the species, but enough to prove informative for this study. The ITS marker, on the other hand, revealed too much variation at this locus to be useful for this study. The high copy number and variation of the ITS gene within individuals made the ITS locus too variable to accurately screen with the SSCP approach in this study. Chu *et al.* (2001) found that the ITS-1 is potentially applicable in the analysis of crustacean population structure, but variations within individuals may obscure population analysis in some cases. The data collected from the ITS gene was too variable and uninformative for this study.

### **Future Work**

In order to gain a better understanding of the population genetic structure of *Uca pugilator* more in depth studies are needed. This study was limited to only analyzing a small fragment of the mitochondrial DNA using the indirect method of SSCP. Future studies should include a larger number of markers and screen both mitochondrial and nuclear DNA. Currently, there are few gene sequences available for use as markers, but with universal primers and DNA sequencing the ability to screen a wide variety of nuclear and mitochondrial genes is possible. DNA sequencing would offer a more powerful tool to detect variation between individuals and offers more statistical power. This would provide better resolution at the population level and a better evolutionary history of the species. Such studies should uncover more variation in the crab's genome and provide a better understanding of the driving forces shaping the genetic structure of this crab.



Increasing the sampling to more Atlantic coast populations and including Gulf coast populations would provide a picture of the population structure of this species throughout its entire range. Increasing the number of populations sampled would provide a better idea on where barriers to dispersal occur for this species. In order to determine if two sibling species are present along the Atlantic coast, more molecular markers, a larger sample size, and more in-depth behavioral and morphological studies of the northern and southern populations are needed. It would also be interesting to compare the population structure of *U. pugilator* to the other sympatric *Uca* species found in this geographic region such as *U. minax* and *U. pugnax*.

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**Table 1.** The collection sites, locations, dates, and totals of all *Uca pugilator* samples collected for this study.

<b>Collection Site</b>	<b>Collector</b>	<b>Latitude/Longitude</b>	<b>Collection Date</b>	<b><i>n</i></b>
<b>Massachusetts</b>				<b>96</b>
Brewster	McLain/Pratt	41 °45.42N 70 °04.58W	7/31/05	52
Dennis	McLain/Pratt	41 °43.54N 70 °12.07W	7/29/05	50
<b>New Jersey</b>				<b>51</b>
Sea Isle City	McLain/Pratt	39 °09.09N 74 °41.52W	7/24/05	51
<b>Virginia</b>				<b>45</b>
Willis Warf	McLain/Pratt	37 °28.35N 75 °51.46W	7/23/05	45
<b>South Carolina</b>				<b>90</b>
Pinckney Island	Weese	32 °14.07N 80 °46.86W	7/6/05	45
Hunting Island	Weese	32 °21.05N 80 °27.55W	7/6/05	45
<b>Georgia</b>				<b>151</b>
Jekyll Island	Weese	31 °01.84N 81 °25.10W	6/19/05	52
Tybee Island	Weese	32 °00.75N 80 °52.16W	6/23/05	49
St. Simon's Island	Weese	31 °10.23N 81 °25.35W	7/7/05	50
<b>Florida</b>				<b>143</b>
Mantanza River	Weese	29 °45.85N 81 °15.68W	7/8/05	50
St. Augustine	Weese	29 °54.62N 81 °18.80W	7/8/05	45
Ft. George Island	Weese	30°24.51N 81 °25.91W	7/8/05	48
<b>Total</b>				<b>576</b>

**Table 2.** 16S rDNA SSCP haplotypes among populations of *Uca pugilator*.

Population	n	Haplotype				
		A	B	C	D	E
<b>1</b>	117	34	75	3	5	0
Brewster, MA	37	6	28	1	2	0
Dennis, MA	44	14	25	2	3	0
Willis Warf, VA	36	14	22	0	0	0
<b>2</b>	119	80	38	1	0	0
Sea Isle City, NJ	45	26	18	1	0	0
Pinckney Island, SC	41	30	11	0	0	0
Hunting Island, SC	33	24	9	0	0	0
<b>3</b>	195	163	25	6	0	1
Tybee Island, GA	47	37	10	0	0	0
St. Simon's Island, GA	34	29	4	1	0	0
Jekyll Island, GA	39	32	4	2	0	1
Ft. George Island, FL	40	34	4	2	0	0
Mantanza River, FL	35	31	3	1	0	0
<b>4</b>	41	41	0	0	0	0
St. Augustine, FL	41	41	0	0	0	0
<b>All Sites</b>	472	318	138	10	5	1



**Table 3.** The frequencies of the 16S rDNA SSCP haplotypes among populations of *Uca pugilator*.

Population	n	Haplotype				
		A	B	C	D	E
<b>1</b>	117	.291	.641	.026	.043	0
Brewster, MA	37	.162	.757	.027	.054	0
Dennis, MA	44	.318	.568	.045	.068	0
Willis Warf, VA	36	.389	.611	0	0	0
<b>2</b>	119	.672	.319	.008	0	0
Sea Isle City, NJ	45	.577	.400	.022	0	0
Pinckney Island, SC	41	.731	.269	0	0	0
Hunting Island, SC	33	.727	.273	0	0	0
<b>3</b>	195	.836	.128	.031	0	.005
Tybee Island, GA	47	.787	.213	0	0	0
St. Simon's Island, GA	34	.852	.118	.029	0	0
Jekyll Island, GA	39	.821	.103	.051	0	.026
Ft. George Island, FL	40	.850	.100	.050	0	0
Mantanza River, FL	35	.886	.086	.029	0	0
<b>4</b>	41	1.00	0	0	0	0
St. Augustine, FL	41	1.00	0	0	0	0
<b>All Sites</b>	472	.674	.292	.021	.011	.002

**Table 4.** Results from a G-test showing significance of haplotypes/genotypes among sites, populations, and sites within populations. For all significant values indicated by  $p < 0.01^*$ ,  $p < 0.001^{**}$ .

<b>Location</b>	<b>G Value</b>	<b>P Value</b>
Between all sites	173.747	0.0000**
Between all Populations	147.896	0.0000**
Among Sites in Population 1	10.205	0.1163
Among Sites in Population 2	4.365	0.3588
Among Sites in Population 3	10.491	.05730
Among Sites in Population 4	N/A	N/A

**Table 5.** Haplotype diversity indices for 16S rDNA among *Uca pugilator*.

<b>Population</b>	<b>Site</b>	<b>Index</b>
<b>1</b>		<b>0.5065 +/- 0.0374</b>
	Brewster, MA	0.4084 +/- 0.0906
	Dennis, MA	0.5825 +/- 0.0545
	Willis Warf, VA	0.4889 +/- 0.0408
<b>2</b>		<b>0.4498 +/- 0.0319</b>
	Sea Isle City, NJ	0.5172 +/- 0.0366
	Pinckney Island, SC	0.4024 +/- 0.0656
	Hunting Island, SC	0.4091 +/- 0.0726
<b>3</b>		<b>0.2853 +/- 0.0388</b>
	Tybee Island, GA	0.3423 +/- 0.0693
	St. Simon's Island, GA	0.2656 +/- 0.0921
	Jekyll Island, GA	0.3212 +/- 0.0919
	Fort George Island, FL	0.2718 +/- 0.0869
	Matanza River, FL	0.2134 +/- 0.0879
<b>4</b>		<b>0.0000 +/- 0.0000</b>
	St. Augustine, FL	0.0000 +/- 0.0000

**Table 6.** 16S rDNA pairwise  $F_{ST}$  values for *Uca pugnator* collection sites above diagonal and geographic distance (km) between sites below diagonal. BR= Brewster, MA; DN= Dennis, MA; SI= Sea Isle City, NJ; WW= Willis Warf, VA; PK= Pinckney Island, SC; HT= Hunting Island, SC; TY= Tybee Island, GA; SS= St. Simon's Island, GA; JK= Jekyll Island, GA; FG= Ft. George Island, FL; SA= St. Augustine, FL; MZ= Mantanza River, FL. \*\* indicates  $p < 0.05$ .

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

**Table 7.** 16S rDNA pairwise  $F_{ST}$  values for *Uca pugilator* populations below diagonal and geographic distance (km) between regions above diagonal. Region 1= Brewster, MA; Dennis, MA; Willis Warf, VA; Region 2= Sea Isle City, NJ; Pinckney Island, SC; Hunting Island, SC; Region 3= Tybee Island, GA; St. Simon's Island, GA; Jekyll Island, GA; Ft. George Island, FL; Mantanza River, FL; Region 5= St. Augustine, FL. All values are significantly significant for  $p < 0.05$ .

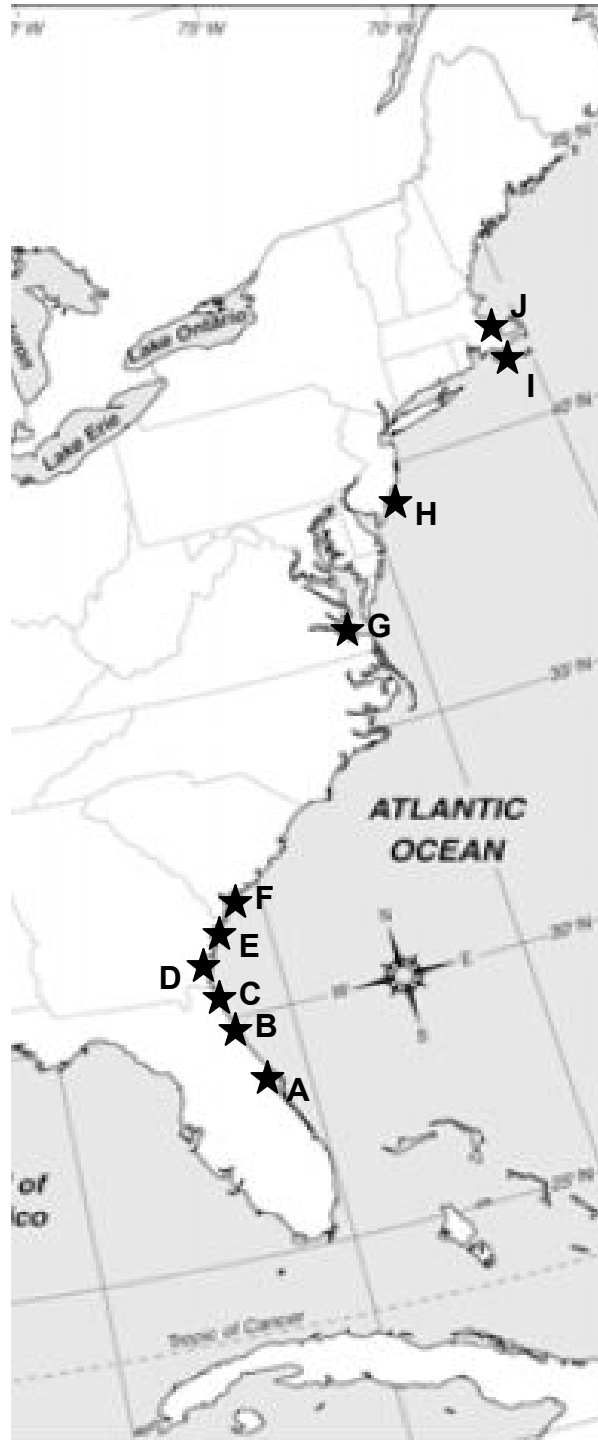
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>1</b>	-	642	1244	1338
<b>2</b>	0.2028	-	648	724
<b>3</b>	0.4308	0.0784	-	62
<b>4</b>	0.5455	0.2280	0.0732	-

**Table 8.** AMOVA analysis of the genetic structure of *Uca pugilator* sites by geographic populations. The north region consists of populations 1 and 2. The south region consists of populations 3 and 4.  $F_{CT}$  is the variance among populations relative to the total variance.  $F_{SC}$  is the variance among sites within populations.  $F_{ST}$  is the variance among sites relative to the total variance.  
 \*\* indicates  $p < 0.05$ .

<b>Divisions</b>	<b>Source of Variation</b>	<b>% Total Variation</b>	<b>F-statistics</b>	<b>P-value</b>
Populations	Among Populations	29.17	0.29169 $F_{CT}$	0.00000**
	Among Sites within Populations	0.61	0.00864 $F_{SC}$	0.00000**
North vs. South	Among Sites	70.22	0.29781 $F_{ST}$	0.00000**
	Among Regions	34.05	0.34050 $F_{CT}$	0.00380**
	Among Sites within Regions	3.51	0.05330 $F_{SC}$	0.00000**
	Among Sites	62.44	0.37565 $F_{ST}$	0.00000*

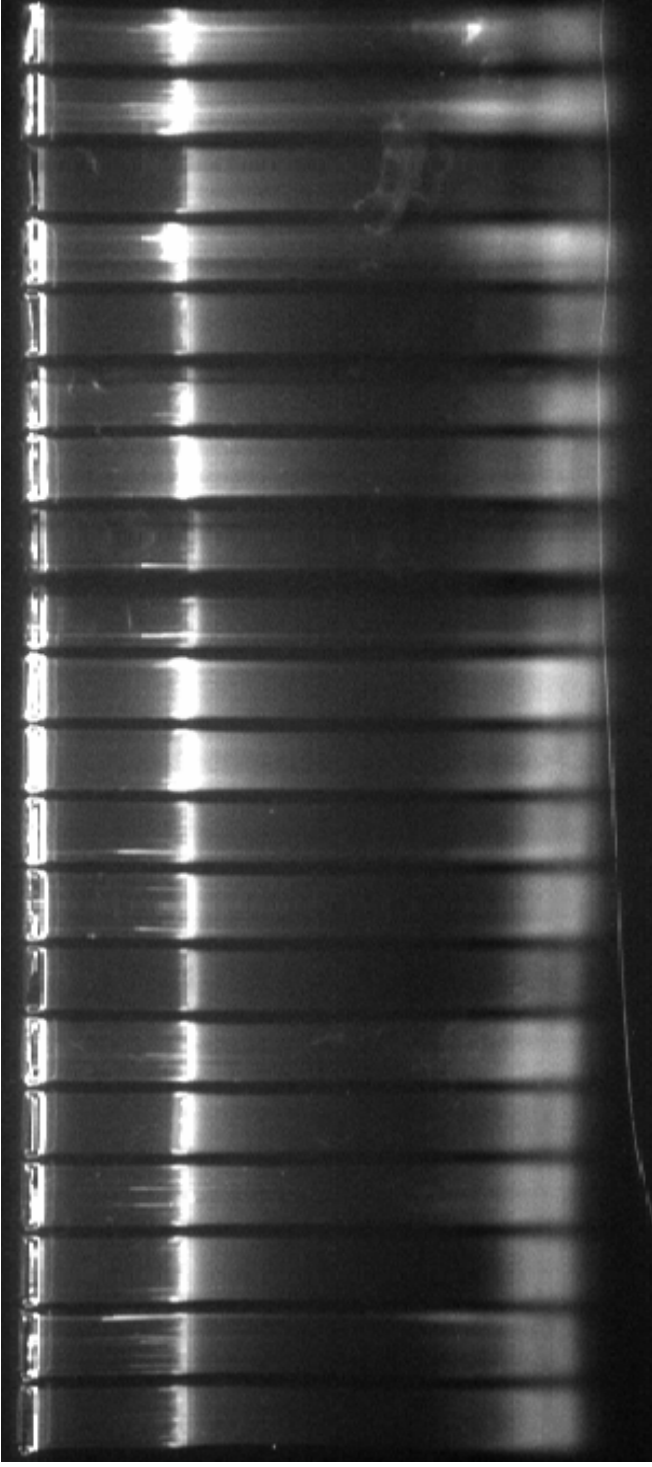
**Table 9.** Matrix of genetic relatedness for 4 populations of *U. pugilator*. Values of Nei's unbiased genetic distance are above the diagonal and for Nei's unbiased genetic identity below. Population 1= Brewster, MA; Dennis, MA; Willis Warf, VA; Population 2= Sea Isle City, NJ; Pinckney Island, SC; Hunting Island, SC; Population 3= Tybee Island, GA; St. Simon's Island, GA; Jekyll Island, GA; Ft. George Island, FL; Mantanza River, FL; Population 4= St. Augustine, FL. All values are statistically significant for  $p < 0.05$ .

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>1</b>	-	0.2677	0.6029	0.8849
<b>2</b>	0.7651	-	0.0411	0.1001
<b>3</b>	0.5472	0.9597	-	0.0118
<b>4</b>	0.4128	0.9048	0.9883	-



**Figure 1.** Collection sites of *Uca pugilator* along the eastern coast of the United States. A) Mantanza River, FL; B) St. Augustine, FL; C) Ft. George Island, FL; D) St. Simon’s Island and Jekyll Island, GA; E) Tybee Island, GA; F) Pinckney Island and Hunting Island, SC; G) Willis Warf, VA; H) Sea Isle City, NJ; I) Dennis MA; J) Brewster, MA.

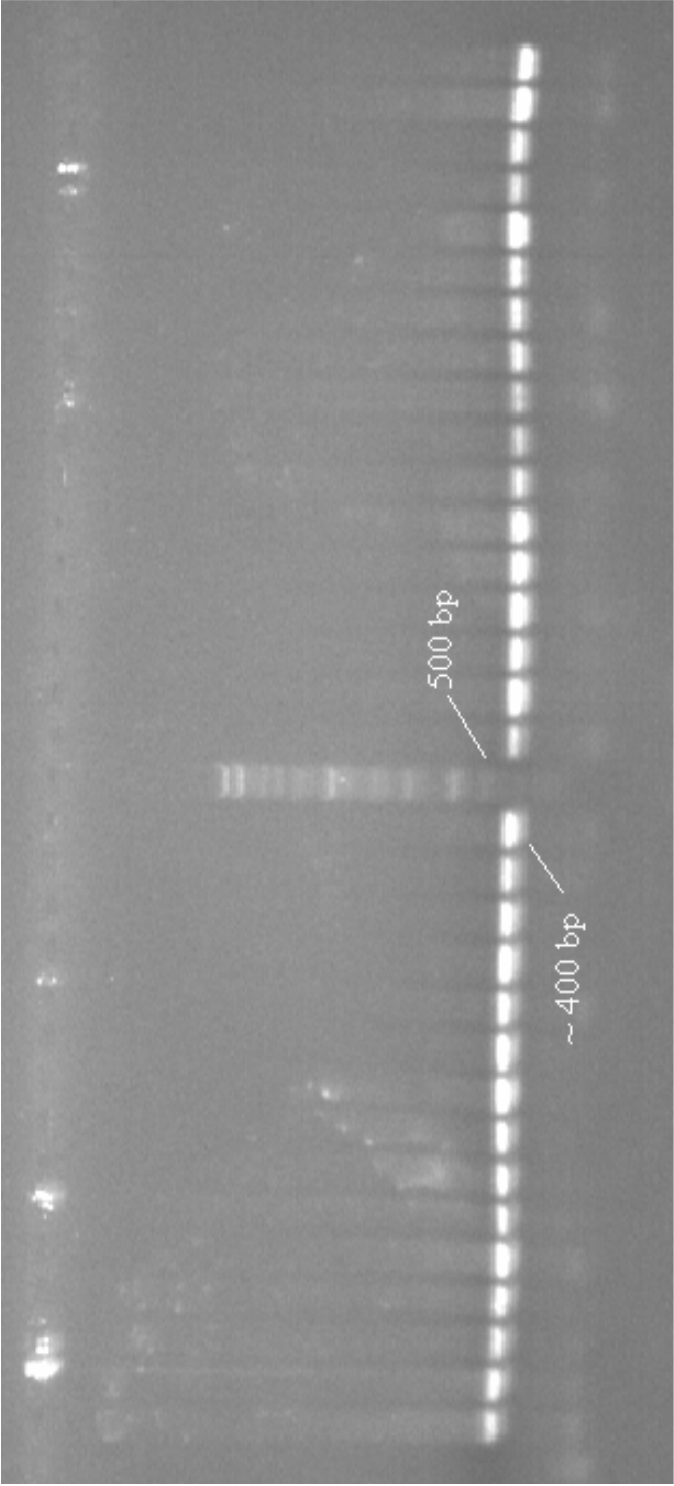




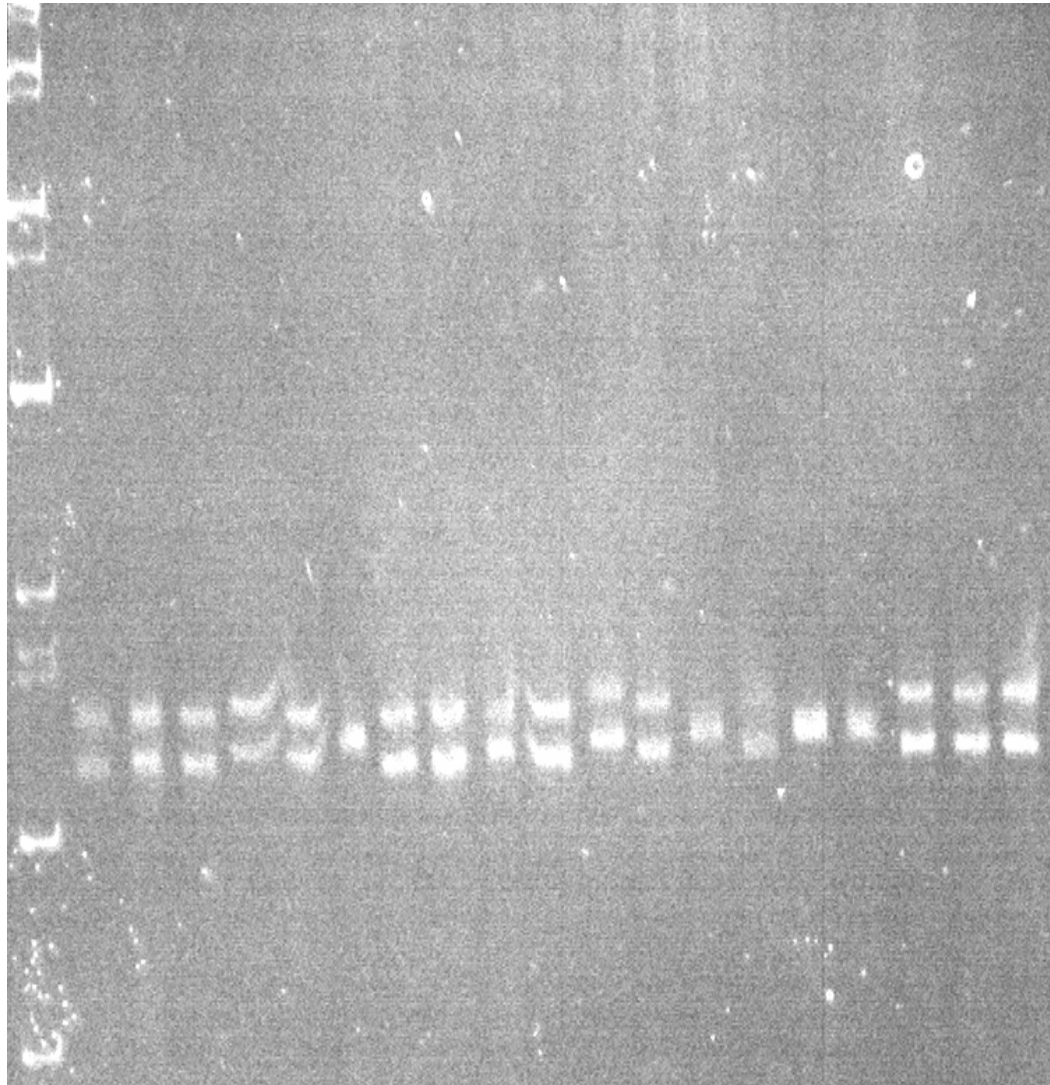
**Figure 2.** CTAB DNA extraction of *Uca pugiator* DNA.



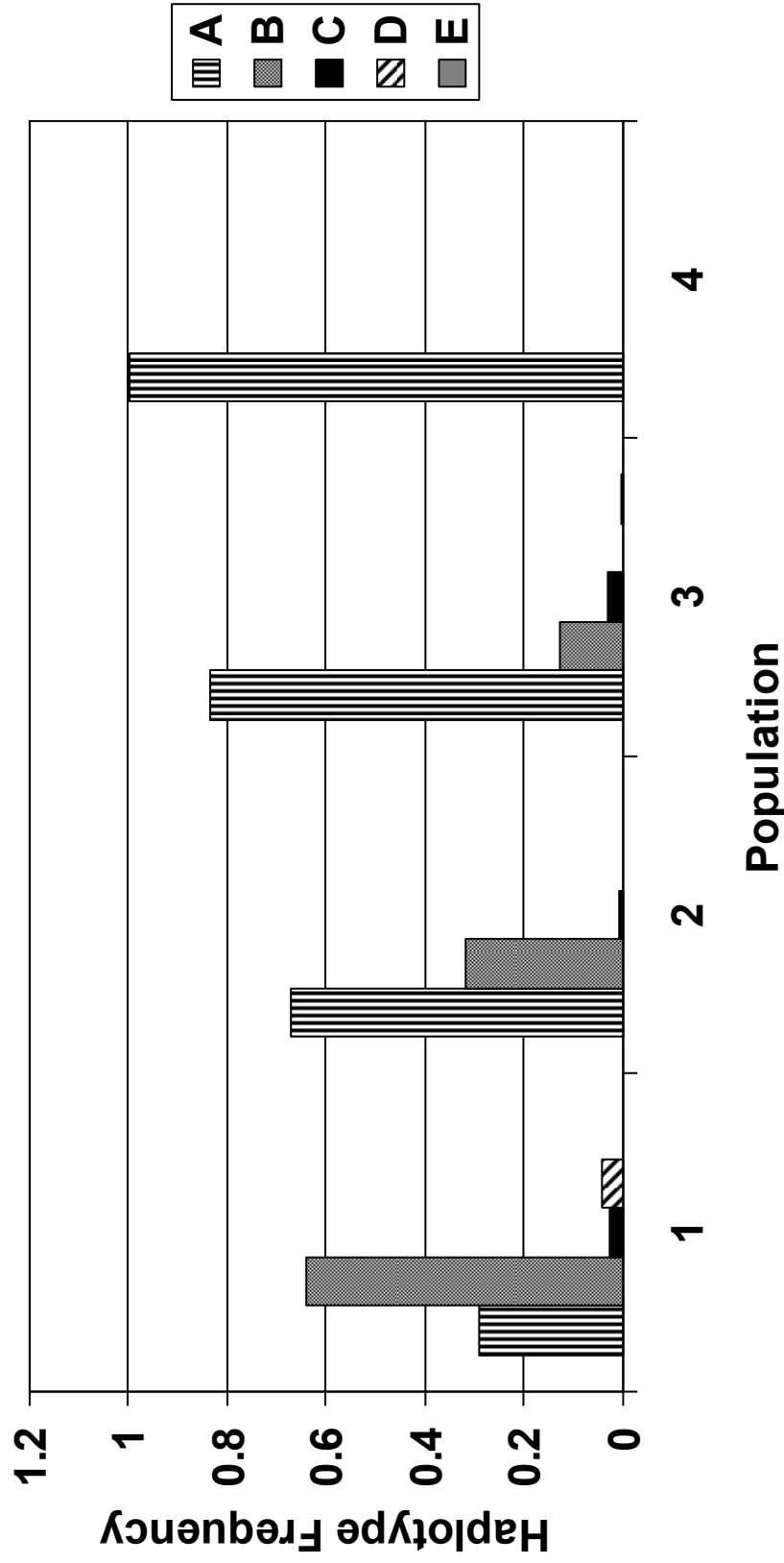
**Figure 3.** PCR amplification of a partial segment of *Uca pugilator* 16S rRNA gene. Lanes 1 and 2 represent negative controls; lane 7 represent the 1 kb molecular marker. Fragment size is about 300 base pairs.



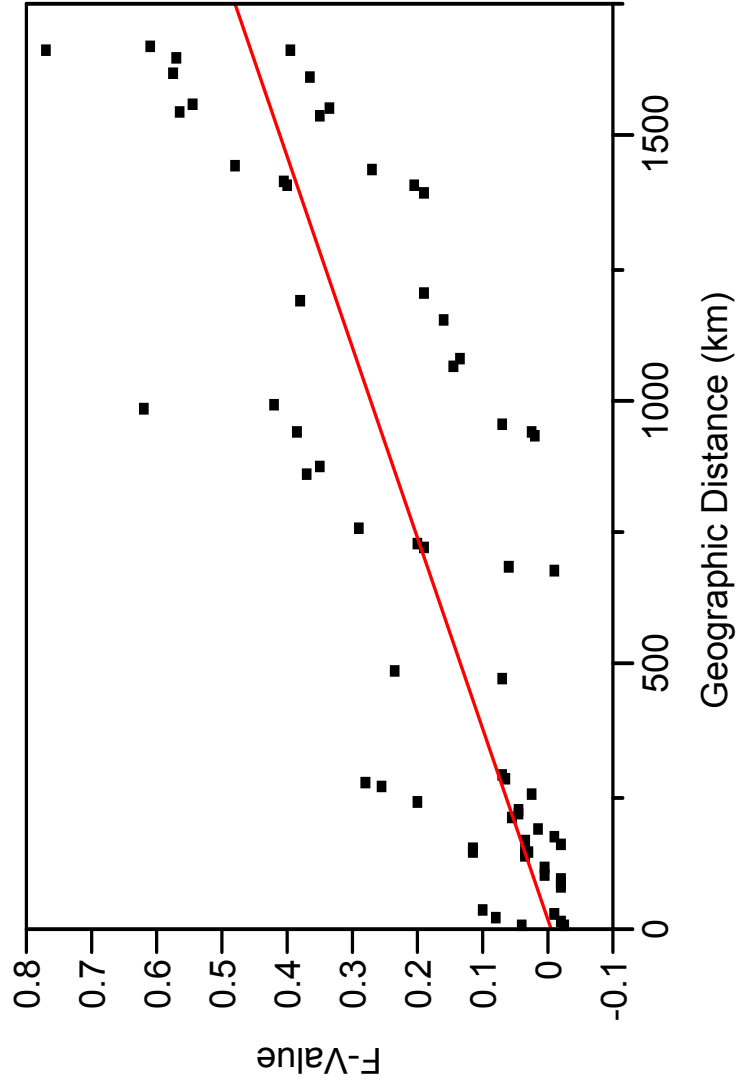
**Figure 4.** PCR amplification of a partial segment of the *Uca pugilator* rDNA internal transcribed spacer 1 (ITS1). Middle lane represents the 1 kb molecular marker; first lane represent the negative control. Amplified fragment is about 400 base pairs.



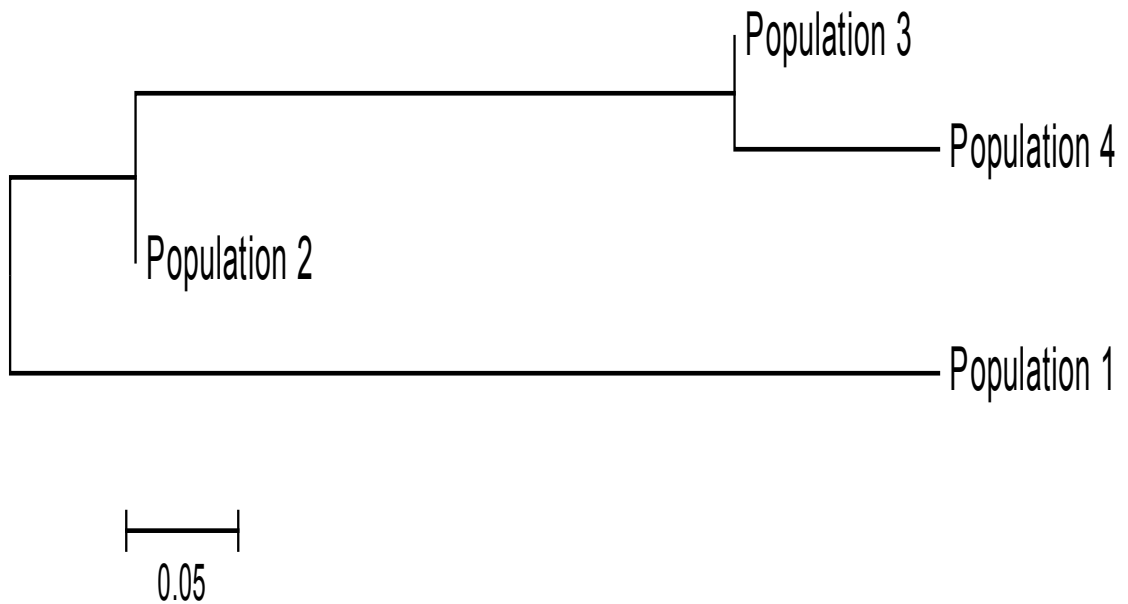
**Figure 5.** Single strand conformation polymorphism of 16S rDNA. Lane 1 represent molecular weight standard. Remaining lanes represent different 16S haplotypes.



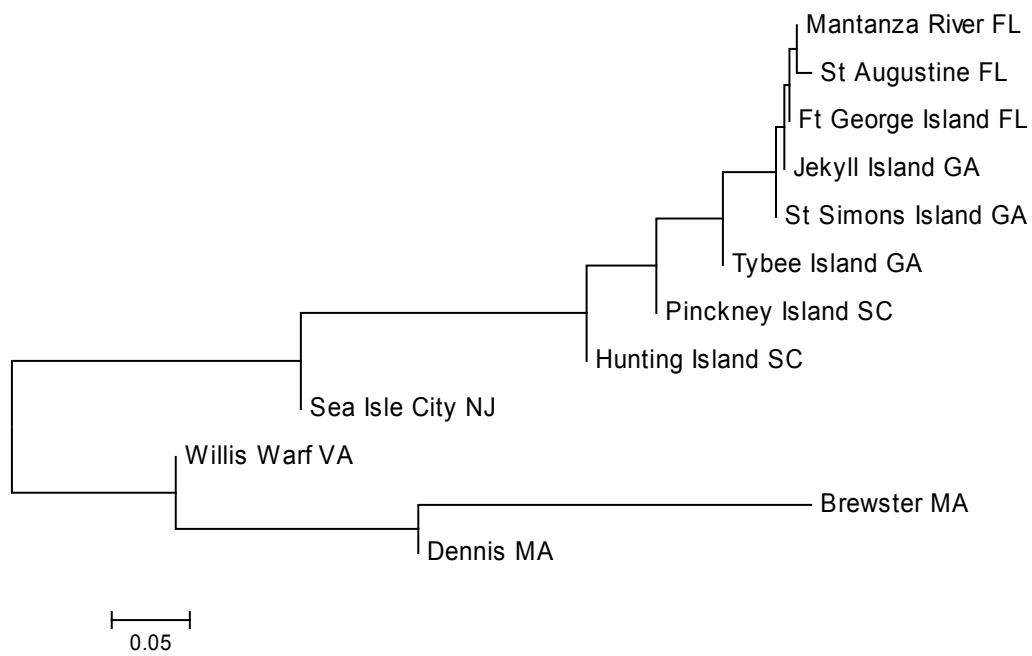
**Figure 6.** Haplotype frequencies for the 16S rDNA for *Uca pugilator* populations occurring along the Atlantic Coast. Population 1= Brewster, MA; Dennis, MA; Willis Warf, VA; Population 2= Sea Isle City, NJ; Pinckney Island, SC; Hunting Island, SC; Population 3= Tybee Island, GA; St. Simon's Island, GA; Jekyll Island, GA; Ft. George Island, FL; Mantanza River, FL; Population 4= St. Augustine, FL.



**Figure 7.** Relationship of  $F_{ST}$  values and geographic distance of collection sites for *Uca pugilator*.  $P < .001$ ;  $r^2 = 0.6448$ .



**Figure 8.** A phenogram calculated from Nei's genetic distance of crabs collected from each population. Population 1= Brewster, MA; Dennis, MA; Willis Warf, VA; Population 2= Sea Isle City, NJ; Pinckney Island, SC; Hunting Island, SC; Population 3= Tybee Island, GA; St. Simon's Island, GA; Jekyll Island, GA; Ft. George Island, FL; Mantanza River, FL; Population 4= St. Augustine, FL.



**Figure 9.** Geographical representation of Nei's genetic distance for 16S rDNA haplotypes of *U. pugilator* sites.