Fine-scale Analysis of Genetic Population Structure Among Horseshoe Crab Spawning Beaches in Delaware Bay.

Final Report

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Abstract

The horseshoe crab, *Limulus polyphemus*, is an important ecological and economic resource throughout the Atlantic coast of North America. Concern over perceived declines in abundance and consequent impacts to shorebird communities has prompted a need for management plans that will preserve this ecosystem relationship. Because effective management regulations require an understanding of population structure, we characterized temporal and spatial genetic variation of spawning *L. polyphemus* adults sampled from 6 beaches in Delaware and New Jersey. We used 12 microsatellite loci to compare allele frequencies of adults sampled during two peak spawning events corresponding to major lunar events within the spawning season (March – June 2004). Results indicate substantial genetic variation over all samples (average H_o = 0.7699, mean number of alleles per collection = 11.74), however minimal (non-significant) differences were observed temporally or spatially among sampling periods or beaches. Correct classification to population of origin by maximum likelihood based assignment tests was only slightly greater than expected by chance alone (49.63% to state, 51.47% to sampling time period). Lack of evidence for genetic population structure suggests that horseshoe crabs within Delaware Bay represent a panmictic population, and can be classified as a single management unit within Delaware Bay.

Introduction

Effective management regulations require an understanding of movement and population dynamics. An interstate fishery management plan was developed in 1998 for the horseshoe crab, *Limulus polyphemus*, by the Atlantic States Marine Fisheries Commission in order to maintain a healthy stock for continued use by humans, migrating shorebirds, or other dependant wildlife. Although several tagging studies have provided insight regarding movement patterns of *L. polyphemus* (Brousseau et al. 2004, Swan et al. 2005), very little information is known pertaining to successful reproduction and population structure. Because harvest of *L. polyphemus* in some regions (such as Delaware Bay) is regulated by multiple agencies, it is necessary important to know if management efforts are focused on a single panmictic population (if adults randomly choose spawning sites), or multiple populations exist. Likewise, it is important to know whether timing of breeding is random or if peak spawning aggregations represent discrete breeding populations.

Limulus polyphemus is a marine invertebrate distributed throughout the Atlantic coast of North America. Inhabiting primarily estuarine and continental shelf habitats for most of the year, adults come ashore to spawn on sandy beaches between March and July (Shuster 1982). Eggs and spawning adults provide an important ecological and economic resource for many organisms. *Limulus polyphemus* eggs are an significant food source for migrating shorebirds, finfish, and loggerhead sea turtles. It was estimated by Castro (1989) that migrating shorebirds gain approximately 40% of their body weight during a 2-3 week stopover in Delaware Bay from feeding on *L. polyphemus* eggs. In addition, *L.*

polyphemus adults are harvested for human use in the medical industry and commercial bait fishery (Shuster 1982).

The status of *L. polyphemus* is poorly understood, although the Delaware Bay appears to support the largest population along the eastern United States (Shuster 1982). Increased exploitation of *L. polyphemus* has caused concern among federal and state agencies, conservation organizations and commercial fishery interests. Threats to *L. polyphemus* include overharvesting by humans, egg consumption by shorebirds, and loss of habitat including beach erosion and shoreline development (ASMFC 1998).

Our objective was to characterize the spatial and temporal genetic population structure of *L. polyphemus* within Delaware Bay. We used 12 microsatellite markers to compare allele frequencies among *L. polyphemus* collected from six different beaches during two different peak spawning events.

Methods

Sample collection

Tissue samples were taken from the claw of adult horseshoe crabs at seven different locations in Delaware Bay (Figure 1). Samples were collected from three New Jersey beaches (Reed's Beach, High's Beach and Fortescue Beach) and three Delaware beaches (Big Stone Beach, Kittshummock, and Fowler's Beach). In addition, samples were collected from an open water trawl within Delaware Bay (April 28, 2004). Approximately 50 samples were collected at each time period and stored in ethanol until DNA extraction could be performed. To examine temporal genetic variation among

breeding adults, each beach was sampled twice corresponding with first and last major lunar events during the spawning season (May 4, and June 1, 2004). Peak spawning events were determined by lunar cycles resulting in extreme low or high tides that correspond to peak spawning activity in horseshoe crabs (Shuster and Botton 1985, Shuster 1982).

Microsatellite analysis

We extracted DNA from claw tissue using a 5% Chelex solution. Tissue was incubated at 55°C in a 5% Chelex solution with 10 ug/mL Proteinase K until digested. After digestion, the mixture was boiled (100°C) for 8 minutes. The resulting supernatant was used to amplify microsatellite DNA loci.

Microsatellite analysis was performed using 12 loci described in King and Eackles (2004): *Lpo*A05, *Lpo*A26, *Lpo*A37, *Lpo*A38, *Lpo*A40, *Lpo*A44, *Lpo*A52, *Lpo*A67, *Lpo*A74, *Lpo*A315, *Lpo*D03, and *Lpo*D06. Each 15 uL PCR reaction consisted of 2.0 μl of genomic DNA extract, 0.875 X PCR buffer (59 mM Tris-HCl, pH 8.3; 15 mM $(NH_4)_2SO_4$; 9 mM β-mercaptoethanol; 6 mM EDTA), 3.75 mM $MgCl_2$, 0.3175 mM each dNTP, 0.07-0.325 μM of each primer (forward primer fluorescently labeled with FAM, NED or HEX; Applied Biosystems, Foster City, CA), 1.3 units of Taq polymerase (Promega Corporation, Madison, WI), and deionized water added to achieve the final volume. The amplification cycle for all loci consisted of an initial denaturing at 94 °C for 2 min; 35 cycles of 94 °C denaturing for 45 sec, 56 °C annealing for 45 sec, 72 °C extension for 2 min; and a 5 min extension at 72 °C. An ABI Prism 3100®™ Genetic Analyzer (Applied Biosystems, Foster City, CA) was used for capillary electrophoresis.

Genotypic data was analyzed and scored with Genescan 3.7.1 Analysis software and Genotyper 3.7 Fragment Analysis software (Applied Biosystems).

Statistical analysis

Genepop (Version 3.2; Raymond and Rousset 1995) was used to test for conformance to Hardy-Weinberg expectations and calculate pairwise estimates of allele frequency differences among collections. Statistical significance for all multiple tests was determined using sequential Bonferroni adjustments (Rice 1989). Genetic distance between collections was summarized using the chord distance of Cavalli-Sforza and Edwards (1967) calculated with BIOSYS-1 (Swofford and Selander 1989). An unrooted tree was fit to the genetic distance matrix using the NEIGHBOR routine from PHYLIP (Felsenstein 1993) and visualized using TreeView (Version 1.6.6; Page 1996). As an additional measure of genetic differentiation, F_{ST} values were generated between each pair of collections. Maximum likelihood assignment tests using GeneClass (Version 1.0.02) were performed to determine the probability of a genotype being classified back into the population from which it was collected (Cornuet et al. 1999). Variables used to classify the data were beach, state, and time of collection (Table 2). Samples collected from the Delaware Bay trawl were analyzed with Structure (Version 2.1; Pritchard et al. 2000) to determine if multiple populations were sampled.

Results

Genotypes were obtained for a total of 586 *L. polyphemus* sampled in the Delaware bay area. Allele frequencies, observed heterozygosity, and the mean number of alleles were estimated for all collections (Table 1). All collections exhibited a high level of genetic diversity as a total of 186 alleles were observed over all 12 loci. Number of alleles per locus ranged from 6 alleles at *Lpo*A315 to 42 alleles at *Lpo*D06. The average number of alleles per collection ranged from 10 (Delaware Bay open water) to 12.58 (High's Beach) and averaged 11.74 alleles. Average observed heterozygosity per collection ranged from 0.751 (Fortescue Beach) to 0.795 (Delaware Bay open water). No deviations from Hardy-Weinberg were observed at any loci in any collection.

No significant spatial or temporal genetic structure was evident among the sampling collections. Maximum likelihood assignment tests did not correctly assign individuals to collection of origin with an incidence any greater than would be expected by chance alone (Table 2). When sampling time period was pooled, individuals were assigned back to collection site (beach) only 16.6 % of the time. When beaches were pooled into state of origin (minus the open water Delaware Bay collection), assignment success rose to 49.63% (Table 2). When individuals from all beaches were pooled into their time period of collection, assignment success was 51.49%.

Pairwise tests of differences in allele frequencies between beaches revealed no significant genetic differentiation. Allelic heterogeneity was not observed among collections (no comparisons were statistically significant, P>.003). Pairwise F_{ST} values located in Table 3 (below diagonal) ranged from -0.0010 (between High's Beach and Fortescue Beach) to 0.0008 (between Kittshummock and Big Stone Beach) and averaged of 0.0000 over all collections. Genetic chord distance values were less than 0.1591

between all populations (Table 3, above diagonal). The greatest distance was between High's Beach and Fowler's Beach and the smallest distance was between Big Stone Beach and Reed's Beach. An unrooted neighbor joining tree depicting the chord distance values illustrates very little differentiation as all populations branch uniformly from several central nodes (Figure 2) with no bootstrap support.

Simulations were performed on the samples collected from the Delaware Bay trawl using Structure (Version 2.1; Pritchard et al. 2000), and samples were assigned to inferred populations. A maximum of six possible populations was assumed based on our sampling from six different beaches. Results of the analysis determined it was most likely that 4 different source populations (K = 4) were contributing samples to the Delaware Bay open water collection. However, the simulations performed only found slight differences between the best fit model (K = 4) and the least likely model (K = 6), indicating very little genetic structure in the sample. In all simulations, allele frequency divergence between inferred populations was 0.000, and sample assignment to inferred populations was random. The probability of each sample being correctly classified into an inferred population decreased as the number of populations (K) increased. In the best fit model (K = 4), the probability of a sample actually belonging to its inferred population was less than 0.256, indicating little support for assignment.

Discussion

Our results indicate that *L. polyphemus* breeding within the Delaware Bay area behave as a large panmictic population with no evidence of philopatry among beaches

sampled. In addition, we found no genetic differences between early breeding adults and those arriving late to beaches.

Our results are consistent with previous investigations of *L. polyphemus* genetic structure along the eastern United States. A microsatellite survey by King et al. (2005) found considerable differentiation rangewide between regions, but very little genetic variation among populations within a region. Earlier studies of mitochondrial DNA and allozymes in *L. polyphemus* also support differentiation at the regional level with considerable genetic differences evident between the Atlantic and Gulf coasts of Florida and the Atlantic coast north of Georgia (Saunders et al 1986, Selander et al 1970). King et al. (2005) proposed four regional groupings or management units within the United States (northeastern, Mid-Atlantic, Atlantic Florida, and Gulf Coast Florida). All of our collection sites fall within the Mid-Atlantic grouping, and our data does not suggest that any further discrimination is necessary within Delaware Bay.

To our knowledge, our study comprises the only investigation of temporal genetic structure among breeding *L. polyphemus*. Due to the correlation of spawning activity with major lunar events, it was important for management considerations to determine if these peak spawning events represented discrete spawning populations. However, our results do not indicate that timing of spawning is an important factor for genetic stock management. Lack of genetic differentiation between early and late breeding *L. polyphemus* suggests that breeding time of an individual may not be consistent from year to year, nor do spawning crabs exhibit fidelity to a particular spawning beach within Delaware Bay. Rather, spawning beach and time may depend on external influences.

The complex life history of *L. polyphemus* may provide explanation as to why so little spatial or temporal structure was observed. *Limulus polyphemus* are long lived organisms (15-20 years) that breed multiple times during their life span, whereas microsatellite statistics assume non-overlapping generations. In addition, there is a large potential for migration and gene mixing since *L. polyphemus* are highly mobile with movements recorded of over 100 km (Swan 2005). Swan (2005) also found *L. polyphemus* migrating into the Delaware Bay from Maryland and other surrounding areas. Individuals may breed up to six times during the spawning season each year (Brousseau et al. 2004) which may also contribute to the genetic mixing of early and late spawning individuals thus preventing temporal variation in allele frequencies.

Management and conservation of *L. polyphemus* is essential due to their significant ecological and economic importance for humans and wildlife. Knowledge of the life history and population dynamics of *L. polyphemus* is imperative for designing appropriate management units and regulations. Our study was designed to investigate the scale at which *L. polyphemus* should be managed in the Delaware Bay area. Our results indicate that *L. polyphemus* within the Delaware Bay area may be managed as one unit regardless of spawning beach and time of spawning.

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Table 1. Sample size (*N*), observed (H_o) and expected heterozygosity (H_e), and number of alleles per locus (N_a) for 12 microsatellite DNA markers for *Limulus polyphemus* sampled from the Delaware Bay area. Average heterozygosities and numbers of alleles are reflected at the end of the table.

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*A05**							
(*N*)	99	71	98	71	94	98	49
177	0.066	0.063	0.036	0.063	0.048	0.041	0.041
181	0.030	0.063	0.036	0.028	0.027	0.036	0.133
183	0.303	0.218	0.255	0.239	0.207	0.286	0.245
185	0.045	0.049	0.071	0.077	0.090	0.087	0.092
187	0.121	0.120	0.112	0.085	0.128	0.122	0.082
189	0.126	0.092	0.122	0.085	0.138	0.107	0.122
191	0.005	0.007	0.015	0.007	0.005	0.015	0.010
193	0.030	0.000	0.020	0.007	0.027	0.015	0.010
195	0.227	0.324	0.255	0.338	0.261	0.245	0.194
197	0.035	0.049	0.046	0.056	0.027	0.031	0.051
199	0.005	0.014	0.026	0.014	0.027	0.000	0.020
201	0.005	0.000	0.005	0.000	0.016	0.015	0.000
H_o	0.869	0.803	0.878	0.775	0.872	0.827	0.776
H_e	0.820	0.817	0.835	0.806	0.845	0.824	0.859
N_a	12	10	12	11	12	11	11
***Lpo*A26**							
(N)	96	69	96	70	94	96	47
147	0.234	0.261	0.224	0.214	0.229	0.214	0.223
149	0.000	0.000	0.005	0.007	0.011	0.005	0.011
151	0.005	0.007	0.010	0.000	0.016	0.005	0.021
153	0.000	0.000	0.000	0.000	0.000	0.005	0.000
155	0.010	0.007	0.010	0.021	0.016	0.010	0.021
157	0.281	0.275	0.354	0.343	0.335	0.281	0.309
159	0.297	0.275	0.292	0.257	0.287	0.359	0.298
161	0.021	0.014	0.031	0.021	0.016	0.016	0.000
163	0.010	0.022	0.000	0.014	0.005	0.026	0.011
165	0.016	0.007	0.005	0.036	0.021	0.010	0.043
167	0.010	0.007	0.005	0.007	0.005	0.005	0.000
169	0.089	0.109	0.052	0.050	0.032	0.047	0.043
171	0.016	0.000	0.005	0.007	0.011	0.010	0.000
173	0.000	0.000	0.000	0.000	0.005	0.000	0.000
175	0.005	0.007	0.005	0.014	0.005	0.005	0.000
177	0.005	0.000	0.000	0.007	0.005	0.000	0.021
185	0.000	0.007	0.000	0.000	0.000	0.000	0.000
H_o	0.708	0.739	0.771	0.729	0.840	0.781	0.745
H_e	0.773	0.773	0.739	0.771	0.754	0.746	0.769
N_a	13	12	12	13	15	14	10

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*A37**							
(N)	99	72	99	70	94	98	49
164	0.343	0.313	0.354	0.371	0.330	0.286	0.316
168	0.111	0.153	0.106	0.129	0.160	0.153	0.102
170	0.000	0.007	0.005	0.000	0.005	0.005	0.000
176	0.131	0.125	0.182	0.150	0.154	0.122	0.173
178	0.359	0.347	0.283	0.293	0.287	0.388	0.398
180	0.040	0.042	0.061	0.057	0.059	0.046	0.010
182	0.010	0.014	0.010	0.000	0.000	0.000	0.000
184	0.005	0.000	0.000	0.000	0.005	0.000	0.000
H_o	0.677	0.792	0.818	0.714	0.755	0.724	0.776
H_e	0.726	0.746	0.751	0.739	0.760	0.731	0.708
N_a	7	7	7	5	7	6	5
***Lpo*A38**							
(N)	99	71	99	71	97	98	48
129	0.020	0.000	0.005	0.007	0.026	0.005	0.000
131	0.005	0.000	0.005	0.000	0.010	0.000	0.000
133	0.035	0.035	0.000	0.000	0.046	0.031	0.010
135	0.379	0.352	0.328	0.345	0.387	0.352	0.396
137	0.293	0.345	0.419	0.352	0.294	0.311	0.260
139	0.020	0.028	0.010	0.014	0.041	0.036	0.010
141	0.010	0.007	0.000	0.021	0.000	0.010	0.010
143	0.040	0.042	0.040	0.070	0.062	0.046	0.073
145	0.141	0.148	0.152	0.134	0.108	0.138	0.177
147	0.025	0.028	0.015	0.021	0.010	0.031	0.031
149	0.000	0.000	0.000	0.000	0.000	0.005	0.010
151	0.025	0.014	0.020	0.021	0.010	0.026	0.021
153	0.005	0.000	0.000	0.014	0.000	0.000	0.000
155	0.000	0.000	0.000	0.000	0.000	0.005	0.000
159	0.000	0.000	0.005	0.000	0.005	0.005	0.000
H_o	0.727	0.746	0.606	0.718	0.773	0.745	0.750
H_e	0.749	0.735	0.695	0.737	0.748	0.758	0.745
N_a	12	9	10	10	11	13	10

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*A40**							
(N)	97	69	94	70	92	97	47
143	0.005	0.007	0.016	0.036	0.027	0.041	0.011
145	0.082	0.051	0.043	0.093	0.033	0.062	0.053
147	0.160	0.210	0.223	0.179	0.196	0.170	0.128
149	0.000	0.007	0.005	0.000	0.005	0.000	0.000
151	0.015	0.014	0.021	0.043	0.027	0.010	0.043
153	0.124	0.109	0.122	0.164	0.109	0.124	0.128
155	0.289	0.268	0.261	0.229	0.239	0.263	0.340
157	0.010	0.007	0.005	0.007	0.005	0.015	0.011
159	0.216	0.196	0.191	0.143	0.266	0.216	0.160
161	0.036	0.014	0.032	0.029	0.016	0.021	0.096
163	0.036	0.051	0.027	0.021	0.011	0.026	0.021
165	0.000	0.007	0.016	0.007	0.027	0.010	0.000
167	0.010	0.014	0.016	0.007	0.005	0.021	0.000
169	0.005	0.022	0.005	0.021	0.011	0.005	0.000
171	0.000	0.007	0.000	0.000	0.005	0.000	0.000
173	0.005	0.007	0.011	0.007	0.016	0.015	0.011
175	0.000	0.000	0.000	0.007	0.000	0.000	0.000
177	0.005	0.007	0.005	0.007	0.000	0.000	0.000
H_o	0.763	0.812	0.745	0.829	0.793	0.814	0.809
H_e	0.823	0.833	0.830	0.861	0.822	0.836	0.820
N_a	14	17	16	16	16	14	11
***Lpo*A44**							
(N)	99	71	99	71	97	98	50
123	0.005	0.007	0.010	0.014	0.005	0.015	0.020
125	0.207	0.197	0.242	0.190	0.206	0.204	0.160
127	0.000	0.000	0.005	0.028	0.000	0.005	0.000
129	0.000	0.000	0.000	0.007	0.010	0.010	0.010
131	0.010	0.021	0.000	0.014	0.010	0.010	0.000
133	0.005	0.000	0.020	0.000	0.015	0.000	0.000
135	0.005	0.000	0.000	0.007	0.005	0.000	0.000
137	0.273	0.289	0.182	0.239	0.196	0.209	0.160
139	0.157	0.225	0.268	0.169	0.175	0.224	0.240
141	0.227	0.148	0.202	0.190	0.263	0.235	0.240
143	0.015	0.070	0.040	0.063	0.052	0.026	0.060
145	0.076	0.028	0.030	0.070	0.062	0.061	0.100
147	0.015	0.014	0.000	0.000	0.000	0.000	0.010
149	0.000	0.000	0.000	0.007	0.000	0.000	0.000
151	0.005	0.000	0.000	0.000	0.000	0.000	0.000
H_o	0.727	0.845	0.707	0.761	0.804	0.745	0.800
H_e	0.804	0.804	0.797	0.837	0.817	0.808	0.828
N_a	12	9	9	12	11	10	9

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*A52**							
(N)	99	72	99	71	95	98	49
156	0.146	0.194	0.121	0.211	0.179	0.168	0.153
158	0.000	0.014	0.005	0.000	0.016	0.005	0.000
160	0.000	0.014	0.010	0.000	0.005	0.000	0.010
162	0.182	0.194	0.187	0.113	0.132	0.122	0.173
164	0.010	0.007	0.005	0.007	0.000	0.020	0.000
166	0.551	0.521	0.576	0.592	0.574	0.571	0.551
168	0.061	0.042	0.045	0.014	0.037	0.056	0.031
170	0.005	0.000	0.010	0.000	0.011	0.000	0.051
172	0.045	0.014	0.035	0.063	0.042	0.051	0.010
174	0.000	0.000	0.005	0.000	0.005	0.000	0.000
176	0.000	0.000	0.000	0.000	0.000	0.005	0.020
H_o	0.626	0.597	0.636	0.535	0.589	0.541	0.653
H_e	0.640	0.655	0.618	0.593	0.621	0.627	0.645
N_a	7	8	10	6	9	8	8
***Lpo*A67**							
(N)	99	71	98	71	97	98	50
84	0.005	0.000	0.000	0.000	0.005	0.000	0.000
90	0.010	0.000	0.000	0.007	0.005	0.015	0.010
92	0.146	0.190	0.102	0.099	0.093	0.138	0.140
94	0.146	0.148	0.138	0.169	0.149	0.153	0.210
96	0.000	0.000	0.005	0.000	0.005	0.000	0.000
98	0.005	0.007	0.020	0.014	0.005	0.026	0.020
100	0.172	0.120	0.128	0.148	0.175	0.117	0.160
102	0.045	0.063	0.082	0.042	0.052	0.061	0.100
104	0.040	0.014	0.046	0.035	0.046	0.036	0.020
106	0.222	0.246	0.281	0.331	0.278	0.255	0.150
108	0.116	0.169	0.117	0.070	0.082	0.092	0.090
110	0.076	0.042	0.077	0.070	0.077	0.087	0.090
112	0.010	0.000	0.000	0.000	0.010	0.010	0.010
114	0.005	0.000	0.005	0.007	0.010	0.005	0.000
116	0.000	0.000	0.000	0.000	0.000	0.005	0.000
118	0.000	0.000	0.000	0.007	0.005	0.000	0.000
H_o	0.899	0.831	0.847	0.831	0.856	0.918	0.820
H_e	0.859	0.838	0.851	0.823	0.847	0.861	0.870
N_a	13	9	11	12	15	13	11

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*A74**							
(N)	97	71	88	70	93	95	48
143	0.005	0.007	0.000	0.000	0.000	0.000	0.000
145	0.263	0.183	0.227	0.243	0.177	0.211	0.229
147	0.052	0.085	0.028	0.057	0.075	0.042	0.083
151	0.005	0.000	0.006	0.014	0.005	0.000	0.000
153	0.021	0.028	0.045	0.043	0.032	0.047	0.010
155	0.010	0.014	0.017	0.014	0.022	0.016	0.021
157	0.113	0.120	0.165	0.093	0.167	0.084	0.156
159	0.041	0.035	0.023	0.029	0.043	0.058	0.063
161	0.010	0.014	0.006	0.000	0.027	0.005	0.000
163	0.361	0.366	0.335	0.371	0.344	0.326	0.313
165	0.103	0.141	0.142	0.114	0.108	0.205	0.125
167	0.010	0.007	0.006	0.021	0.000	0.005	0.000
171	0.005	0.000	0.000	0.000	0.000	0.000	0.000
H_o	0.784	0.761	0.818	0.786	0.860	0.832	0.875
H_e	0.776	0.794	0.789	0.780	0.805	0.796	0.807
N_a	13	11	11	10	10	10	8
***Lpo*A315**							
(N)	99	72	99	71	97	98	50
135	0.005	0.014	0.010	0.007	0.015	0.005	0.000
137	0.328	0.361	0.338	0.303	0.314	0.321	0.280
145	0.500	0.431	0.495	0.535	0.510	0.526	0.490
147	0.167	0.181	0.157	0.148	0.160	0.148	0.230
149	0.000	0.000	0.000	0.007	0.000	0.000	0.000
151	0.000	0.014	0.000	0.000	0.000	0.000	0.000
H_o	0.576	0.556	0.667	0.592	0.588	0.571	0.760
H_e	0.618	0.656	0.619	0.604	0.618	0.602	0.635
N_a	4	5	4	5	4	4	3
***Lpo*D03**							
(N)	99	72	98	71	96	98	50
131	0.000	0.000	0.010	0.007	0.000	0.005	0.000
135	0.146	0.167	0.179	0.211	0.167	0.173	0.120
139	0.076	0.063	0.061	0.035	0.073	0.092	0.090
143	0.384	0.375	0.393	0.380	0.359	0.306	0.370
147	0.056	0.021	0.020	0.042	0.063	0.041	0.070
151	0.035	0.063	0.056	0.077	0.068	0.041	0.060
155	0.056	0.097	0.107	0.070	0.052	0.077	0.040
159	0.015	0.028	0.000	0.021	0.026	0.026	0.010
163	0.146	0.104	0.102	0.092	0.120	0.168	0.140
167	0.035	0.021	0.010	0.021	0.021	0.010	0.020
171	0.035	0.035	0.046	0.021	0.010	0.031	0.040
175	0.005	0.028	0.000	0.007	0.031	0.026	0.020

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*D03 continued**							
179	0.010	0.000	0.015	0.014	0.010	0.005	0.020
H_o	0.848	0.847	0.776	0.817	0.792	0.806	0.820
H_e	0.798	0.805	0.786	0.792	0.814	0.832	0.816
N_a	12	11	11	13	12	13	12
***Lpo*D06**							
(N)	99	72	99	70	95	98	50
140	0.005	0.000	0.005	0.000	0.000	0.005	0.000
152	0.000	0.007	0.005	0.000	0.000	0.000	0.000
156	0.005	0.000	0.010	0.000	0.016	0.010	0.020
160	0.010	0.014	0.010	0.000	0.011	0.000	0.020
164	0.000	0.000	0.010	0.000	0.005	0.010	0.000
168	0.005	0.000	0.005	0.007	0.000	0.000	0.000
172	0.005	0.028	0.005	0.014	0.016	0.000	0.000
176	0.020	0.007	0.025	0.014	0.011	0.020	0.030
180	0.015	0.063	0.010	0.043	0.016	0.056	0.030
184	0.136	0.146	0.141	0.093	0.147	0.097	0.120
188	0.146	0.153	0.157	0.150	0.147	0.153	0.130
192	0.162	0.139	0.126	0.129	0.137	0.102	0.130
196	0.141	0.042	0.096	0.093	0.068	0.082	0.090
198	0.000	0.000	0.000	0.000	0.000	0.010	0.000
200	0.056	0.035	0.040	0.043	0.026	0.051	0.080
204	0.025	0.069	0.020	0.029	0.011	0.041	0.050
208	0.040	0.014	0.015	0.007	0.021	0.010	0.020
212	0.010	0.021	0.005	0.014	0.021	0.020	0.030
216	0.005	0.007	0.005	0.014	0.005	0.010	0.010
218	0.000	0.000	0.000	0.000	0.005	0.000	0.000
220	0.010	0.007	0.015	0.007	0.005	0.026	0.000
222	0.005	0.014	0.010	0.007	0.000	0.000	0.000
224	0.000	0.007	0.005	0.014	0.000	0.000	0.000
226	0.005	0.007	0.010	0.000	0.005	0.005	0.010
228	0.005	0.000	0.000	0.000	0.000	0.005	0.000
230	0.010	0.007	0.010	0.014	0.016	0.005	0.010
232	0.000	0.000	0.000	0.007	0.005	0.000	0.010
234	0.010	0.021	0.015	0.021	0.011	0.015	0.010
238	0.030	0.042	0.066	0.029	0.053	0.041	0.040
242	0.040	0.035	0.051	0.064	0.068	0.056	0.070
246	0.020	0.028	0.056	0.057	0.063	0.051	0.050
248	0.000	0.000	0.000	0.000	0.005	0.000	0.000
250	0.025	0.042	0.040	0.043	0.047	0.046	0.020
252	0.000	0.000	0.000	0.000	0.000	0.005	0.000
254	0.025	0.028	0.010	0.050	0.032	0.036	0.000
258	0.015	0.007	0.010	0.007	0.021	0.005	0.000
262	0.005	0.000	0.010	0.014	0.005	0.020	0.020
266	0.005	0.007	0.000	0.000	0.000	0.000	0.000

Locus	Big Stone	Fowler's	Kitts hummock	Fortescue	High's	Reed's	Delaware Bay
***Lpo*D06 continued**							
270	0.000	0.000	0.000	0.007	0.000	0.000	0.000
272	0.000	0.000	0.000	0.000	0.000	0.005	0.000
276	0.000	0.000	0.000	0.007	0.000	0.000	0.000
280	0.000	0.007	0.000	0.000	0.000	0.000	0.000
H_o	0.899	0.903	0.889	0.929	0.905	0.888	0.960
H_e	0.907	0.922	0.919	0.931	0.920	0.934	0.930
N_a	30	28	31	28	29	29	22
Mean							
H_o	0.759	0.769	0.763	0.751	0.786	0.766	0.795
(S.E.)	0.030	0.029	0.027	0.031	0.029	0.033	0.022
H_e	0.774	0.782	0.769	0.773	0.781	0.78	0.786
(S.E.)	0.024	0.022	0.026	0.028	0.026	0.027	0.026
N_a	12.42	11.33	12.00	11.75	12.58	12.08	10.00
(S.E.)	1.83	1.74	1.92	1.78	1.79	1.79	1.33

Table 2. Maximum likelihood assignment success (Cornuet et al. 1999) for *Limulus polyphemus* collected from six beaches in the Delaware Bay area.

Variable	Individuals grouped by:	Total # groups compared	% Correct classification	# Correctly classified
Beach and Time	Beach and time period separate	12	7.84%	42 / 536
Beach	Beach, time periods pooled	6	16.60 %	87 / 536
Time	Time, beaches pooled	2	51.47 %	276 / 536
State and Time	State and time, beaches pooled within state	4	24.81 %	133 / 536
State	State, time periods and beaches pooled	2	49.63 %	266 / 536

Table 3. Matrix indicating distance coefficients and F_{ST} values for *Limulus polyphemus* collected in the Delaware Bay area. Numbers above the diagonal are Cavalli-Sforza and Edwards (1967) chord distance. Numbers below the diagonal are pairwise F_{ST} estimates. For comparisons, collections are grouped by beach and include samples from both time periods.

	Collection	BS	FW	KH	FT	HI	RE
1	Big Stone Beach (BS)	*****	0.1416	0.1348	0.1434	0.1339	0.1286
2	Fowler's Beach (FW)	-0.0004	*****	0.1502	0.1591	0.1591	0.1518
3	Kittshummock (KH)	0.0008	0.0001	*****	0.1447	0.1335	0.1420
4	Fortescue Beach (FT)	0.0003	-0.0001	-0.0007	*****	0.1525	0.1420
5	High's Beach (HI)	-0.0002	0.0009	-0.0003	-0.0010	*****	0.1364
6	Reed's Beach (RE)	-0.0008	0.0003	0.0007	0.0003	-0.0003	*****

Figure 1. Sampling locations of *Limulus polyphemus* collected from Delaware Bay in 2004.

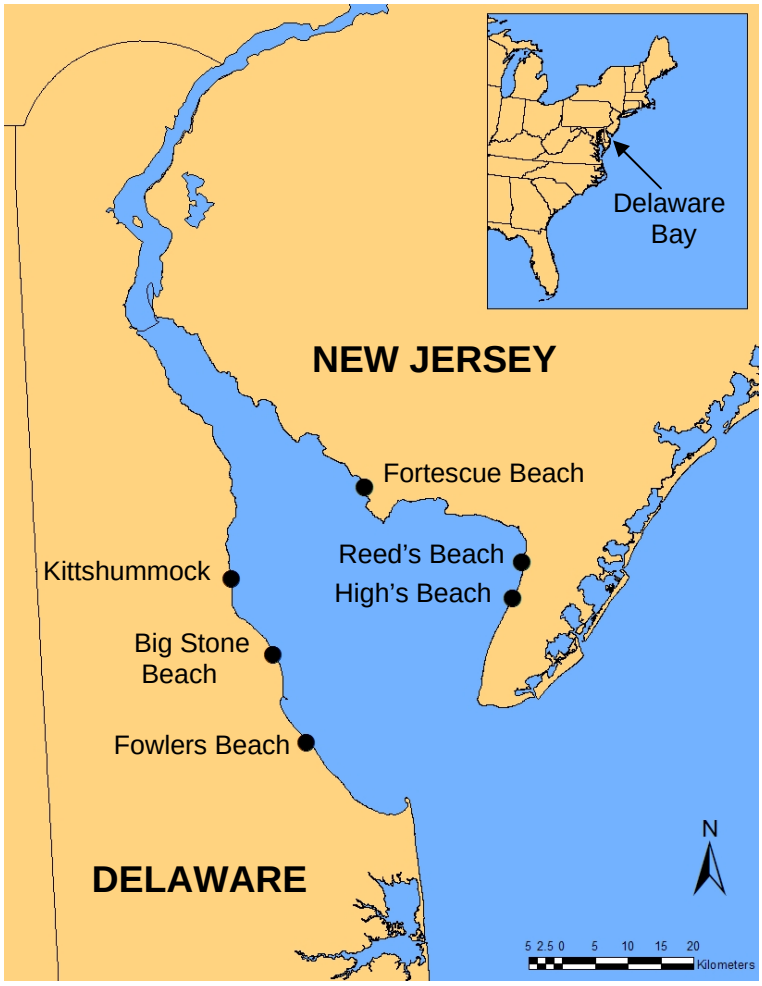
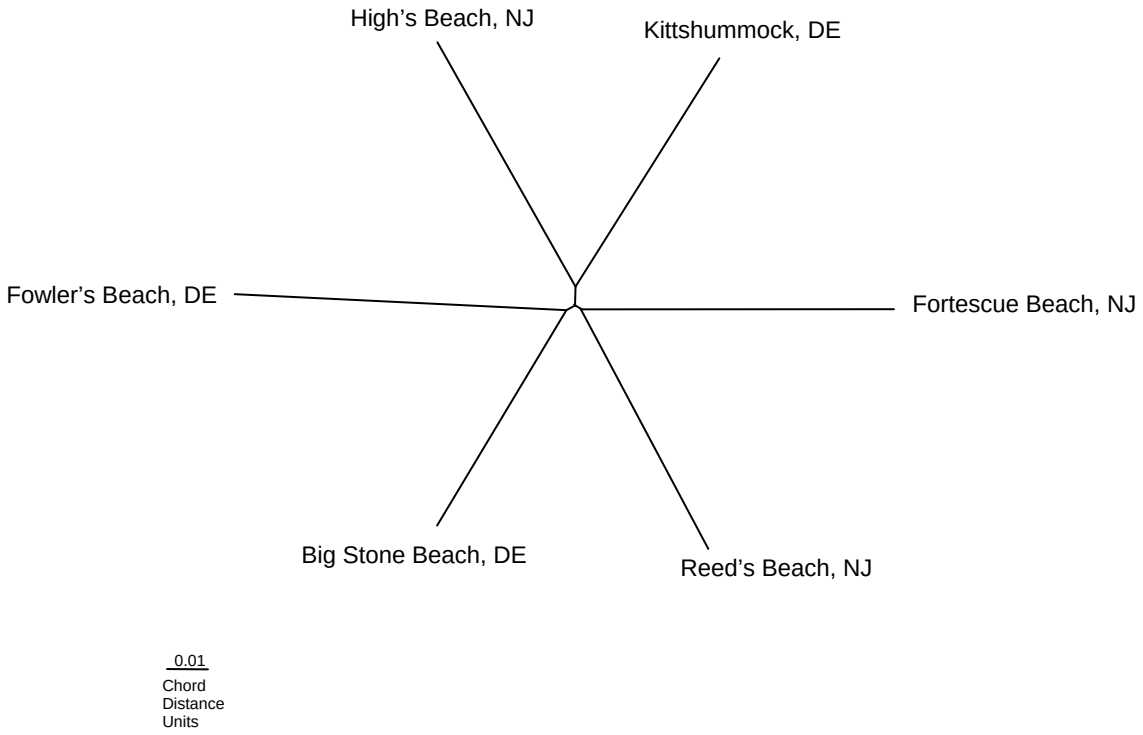
Figure 2. Unrooted neighbor joining tree depicting genetic distance (chord, Cavalli-Sforza and Edwards 1967) among 6 *Limulus polyphemus* collections sampled from the Delaware Bay area. No bootstrap estimates are associated with any nodes. Due to lack of evidence for temporal genetic structure, samples collected throughout the 2004 spawning season were pooled by collection location.