Extreme Population Subdivision in the Crown Conch (Melongena corona): Historical and Contemporary Influences

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Abstract

Organisms with crawl-away larvae are thought to experience highly restricted gene flow. Here, we assess the pattern and magnitude of population subdivision of the direct developing snails in the Melongena corona complex and assess the validity of species and subspecies designations in the genus. A total of 516 individuals from 15 locations were assayed at 8 microsatellite loci. Levels of genetic diversity were moderate and typical of gastropods. There were from 8 to 28 alleles per locus and the average observed per sample heterozygosity ranged from 0.16 to 0.79. Levels of genetic divergence were generally large with all sample pairwise $F_{ST}$ values statistically significant and ranging from 0.011 to 0.438 and Jost’s $D_{EST}$ ranging from 0.028 to 0.731. A Bayesian analysis identified 7 clusters of, usually adjoining, samples. The population subdivision is likely derived from a complex mixture of life-history attributes, frequent short-distance dispersal via swimming larvae, rare short- and long-distance dispersal of rafting larvae and eggs, and a patchwork of adjacent and adjoining habitats. As with a previous study, the current taxonomy is not supported by the genetic results and the complex can be considered as M. corona, a single, albeit clearly geographically genetically structured, species.

Key words: crawl-away larvae, Florida, Intracoastal Waterway, microsatellite loci, rafting

An understanding of evolutionary processes that lead to population subdivision and speciation requires knowledge of the roles played by a diverse array of factors, such as dispersal and localized adaptation (Riginos et al. 2011). In general, population structure of marine organisms results from the interplay between a complex series of contemporary and historical events (Grosberg and Cunningham 2001; Weetman et al. 2006). Marine populations are commonly assumed to be highly linked by dispersal and the absence of obvious physical barriers. Often, populations of species with low dispersal potential are expected to be more divergent and to differentiate faster than those with higher vagility (Scheltema 1971, 1986; Burton and Feldman 1982; Collin 2001). Several studies have investigated the link between realized and potential gene flow in a variety of species (Hellberg 1996; Kyle and Boulding 2000; Rocha et al. 2002; Ayre et al. 2009). In general, species with direct-developing, demersal larvae exhibit highly restricted gene flow over moderate to large geographic scales and those with long-lived planktonic larvae show little to no structure over similar ranges (Hedgecock 1986; Scheltema 1986; Janson 1987; Armdt and Smith 1998). It also has been noted that population structure in species with restricted dispersal may reflect historical barriers to gene flow, whereas structure in more vagile species reflects modern day barriers (Pelc et al. 2009). In contrast, a few studies have shown little to no relationship between pelagic larval duration and population structure (Weersing and Toonen 2009; Riginos et al. 2011). For example, in their examination of Littorina spp., Kyle and Boulding (2000) found that one direct-developing species from the northeastern Pacific exhibited similar levels of marked population subdivision as a congeneric planktotrophic species where another direct developer exhibited no significant population subdivision. Ayre et al. (2009) examined several species of marine invertebrates with distributions spanning two recognized biogeographic regions in southeastern Australia. Assaying genetic subdivision in several species of barnacle, chiton, limpet, periwinkle, whelk, and starfish, which included both direct-developing larvae (one whelk and one starfish) as well as planktonic larva (all others), they found that the habitat specificity of the species was more important than larval type in
determining population subdivision. The disparity between potential and realized gene flow in a number of these species may be explained by suitable habitat, direction and velocity of regional ocean currents, and natural selection (Riginos and Nachman 2001).

In examining the various patterns of population subdivision in marine taxa in the southeastern United States, a few studies have addressed direct-developing taxa (e.g., Berlocher 2000; Wise et al. 2004), whereas most have focused on species with high dispersal potential (e.g., Karl and Avise 1992; Schulze et al. 2000; Hare and Weinberg 2005). It is often assumed that genetic barriers among populations of direct-developing species arise as a result of the limits to dispersal. Based on stereotypical direct development and narrow habitat preferences, it is assumed that the snails in the genus Melongena (crown conches; Gmelin 1791) likely are highly structured (Clench and Turner 1956; Tucker 1994). Snails in the genus Melongena are strongly philopatric, and in the southeastern United States, they inhabit contiguous intertidal areas interspersed with stretches of unsuitable habitat. Over evolutionary time, this situation may be expected to produce large numbers of ecologically similar but genetically distinct populations. The differences in shell morphologies (e.g., color and spination patterns) among populations of crown conchs have been interpreted as an indicator of underlying genetic differentiation resulting in the genus being composed of several species and subspecies (Figures 1 and 2).

Presently, there are 3 recognized species in what is referred to as the Melongena corona complex (Clench and Turner 1956; Tucker 1994): M. sprucecreekensis Tucker 1994, M. bicolor (Say 1826), and M. corona (Gmelin 1791), which in turn is divided into 3 subspecies, M. c. johnstonei Clench and Turner 1957, M. c. corona (Gmelin 1791), and M. c. altispira Pilsbry and Vanatta 1934 (Figure 2). Recent mitochondrial DNA analyses, however, do not support these designations (Hayes and Karl 2009) revealing levels of sequence divergence far below those found in homologous sequences from other related species (Thomaz et al. 1996; Holland and Hadfield 2002; Hasse et al. 2003). Hayes and Karl (2009) conclude that all the taxonomic divisions and subdivisions in the corona complex should be subsumed as M. corona. Even so, this conclusion was based solely on mitochondrial DNA and may reflect other processes, such as hybridization and introgression, not taxonomic status. Given the extremely low dispersal ability of this species, it would also be interesting to know how and to what extent nuclear genes in M. corona show population subdivision throughout its range and, if present, does the pattern correspond to the current taxonomy.

**Materials and Methods**

**Sample Collection and Genotyping**

From July 1999 through May 2003, mature conchs were collected in the intertidal zone throughout their range in Florida and Alabama (Figure 2; Table 1). At each location, an attempt was made to collect at least 40 individuals. Once in the lab, all snails were anesthetized using 7% MgCl₂ in filtered seawater, a portion of the foot tissue was removed and stored in 95% ethanol, and the remainder frozen at −20 °C until DNA extraction was performed. Total cell DNA (tDNA) was isolated using either a phenol/chloroform method modified from Ausubel et al. (1993; frozen tissue), a modified chelex-based protocol (Estoup et al. 1996; ethanol-preserved tissue), a salt extraction protocol (Simison and Lindberg 1999; ethanol-preserved tissue), or the Wizard Genomic DNA Purification Kit (Promega, Madison, WI; samples recalcitrant to other methods). Purified tDNA was dried and resuspended in 50 μl of 1× TLE (10 mM Tris–HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0) and stored at −20 °C. Individuals were genotyped at 8 microsatellite loci following Hayes and Karl (2004). After amplification, the 8 loci were combined into 2 different mixes: mix 1—McoA4, Mco6, Mco10, and Mco12 and mix 2—Mco2, Mco3, McoE4, and Mco5. Depending on DNA concentration, 0.25–2.00 μl of each amplification were combined in 12 μl of deionized...
formamide containing 0.5 ml of GeneScan-500 size marker (Applied Biosystems, Carlsbad, CA) and denatured for 3 min at 95 °C. Samples were subjected to capillary electrophoresis on an ABI Prism 310 Genetic Analyzer running DATA COLLECTION software (ver. 1.2.2) and GENESCAN analysis software (ver. 3.1.2; Applied Biosystems). Alleles were sized using the GENESCAN local Southern method (Ghosh et al. 1997), and all genotypes were entered into a MICROSOFT ACCESS 2000 database (Microsoft Corp., Redmond, WA).

Statistical Analyses

All possible loci by population pairs were tested for deviations from Hardy–Weinberg equilibrium (HWE) using GENEPOP 3.1b (Raymond and Rousset 1995) and the Markov chain method for unbiased estimates of statistical significance (Guo and Thompson 1992). Parameters for all tests were set to 10 000 dememorization steps and 1000 batches with 10 000 iterations per batch. Genotypic linkage disequilibrium was also tested in GENEPOP 3.1b using a ratio test with parameters set to 10 000 dememorization steps and 1000 batches with 10 000 iterations per batch (Raymond and Rousset 1995; Slatkin and Excoffier 1996). Number of alleles, allele frequencies, allele size range, observed and expected heterozygosity, and the variance in repeat number were calculated with MICROSATELLITE ANALYZER (Dieringer and Schlötterer 2003). Average gene diversity per locus across populations was estimated.

Table 1  Collection sites and sample sizes (N) used in this study

<table>
<thead>
<tr>
<th>Collection Site (Abbreviation)</th>
<th>Location</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange Beach, AL (OB)</td>
<td>30.24N, 87.74W</td>
<td>35</td>
</tr>
<tr>
<td>Big Lagoon, FL (BL)</td>
<td>30.31N, 87.40W</td>
<td>30</td>
</tr>
<tr>
<td>Saint Joseph Peninsula, FL (SJP)</td>
<td>29.77N, 85.40W</td>
<td>46</td>
</tr>
<tr>
<td>Panacea, FL (PA)</td>
<td>29.98N, 84.38W</td>
<td>40</td>
</tr>
<tr>
<td>Cedar Key, FL (CK)</td>
<td>29.16N, 83.03W</td>
<td>40</td>
</tr>
<tr>
<td>Tampa Bay, FL 1 (TB1)</td>
<td>27.65N, 82.68W</td>
<td>9</td>
</tr>
<tr>
<td>Tampa Bay, FL 2 (TB2)</td>
<td>27.87N, 82.61W</td>
<td>12</td>
</tr>
<tr>
<td>Tampa Bay, FL 3 (TB3)</td>
<td>27.97N, 82.56W</td>
<td>20</td>
</tr>
<tr>
<td>Pine Island, FL (PI)</td>
<td>26.66N, 82.15W</td>
<td>30</td>
</tr>
<tr>
<td>East Cape Sable, FL (ECS)</td>
<td>25.13N, 81.08W</td>
<td>40</td>
</tr>
<tr>
<td>Big Torch Key, FL (BT)</td>
<td>24.72N, 81.45W</td>
<td>29</td>
</tr>
<tr>
<td>Barnes Sound, FL (BS)</td>
<td>25.19N, 80.42W</td>
<td>25</td>
</tr>
<tr>
<td>Matheson Hammock, FL (MH)</td>
<td>25.68N, 80.26W</td>
<td>23</td>
</tr>
<tr>
<td>Lake Worth Cove, FL (LW)</td>
<td>26.83N, 80.05W</td>
<td>10</td>
</tr>
<tr>
<td>Merritt Island Banana River, FL (MIB)</td>
<td>28.36N, 80.65W</td>
<td>42</td>
</tr>
<tr>
<td>Spruce Creek Estuary, FL (PCA)</td>
<td>29.10N, 80.97W</td>
<td>54</td>
</tr>
<tr>
<td>Flagler Beach, FL (FB)</td>
<td>29.48N, 81.14W</td>
<td>31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>516</strong></td>
</tr>
</tbody>
</table>

Figure 2. Geographic distribution and sampling locations for Melongena spp. in this study. Location abbreviations are as in Table 1. Heavy black lines indicate the species range. Subpopulations defined by STRUCTURE are indicated with ovals surrounding sample sites. Locations not included in ovals are sites where Q values were low and ancestries were mixes of 2 or more of the defined subpopulations with arrows indicating the population affinities.
with FSTAT version 2.9.3 (Goudet 1995, 2001), and the relationship between allelic diversity and sample size was tested with a Spearman’s rank correlation using JMP (Version 8.0.2.2; SAS Institute Inc., Cary, NC).

Recently, it was noted that Wright’s $F_{ST}$ (Wright 1951) is a measure of fixation not differentiation as it is often used (Hedrick 2005; Jost 2008; Bird et al. 2011; Meirmans and Hedrick 2011). Because of intrasample variation, $F_{ST}$ estimates can be considerably smaller than 1.0 even when 2 samples share no alleles. Jost (2008) developed a metric, $D_{EST}$, which was shown to be a robust measure of population differentiation (but see Bird et al. 2011). Unfortunately, methods for combining multiple loci and for testing statistical significance of $D_{EST}$ are controversial and not fully developed. Here, we use $F_{ST}$ and $D_{EST}$ to measure the genetic distance between all pairwise samples. Jost’s $D_{EST}$ was calculated using GENODIVE 20b.20 (Meirmans and Van Tienderen 2004), and $F_{ST}$ values and significance levels were calculated using ARLEQUIN 3.5 (Excoffier and Lischer 2010). To further evaluate the pattern of population subdivision, we ran the program STRUCTURE 2.3.2 (Pritchard et al. 2000; Hubisz et al. 2009) using an admixture model with collection sites as a location prior (LOCPRIOR) and correlated allele frequencies, 50 000 burn-in steps, and 500 000 data collection steps with the number of subpopulations (i.e., $k$) ranging from 1 to 15. To assess whether the analysis had converged, we ran 5 independent runs for each $k$. The most likely value of $k$ was determined using the $\Delta k$ method of Evanno et al. (2005).

The magnitude and direction of migration ($N_m$) among all sample sites was estimated using the program MIGRATE (Beerli and Felsenstein 2001). A Bayesian analysis with one long chain of 500 000 steps was run with 10 000 steps discarded as a burn-in. Estimated effective sample sizes and unimodality of the posterior distributions were used to determine if the analysis had converged on the most likely estimates, and the estimates from 2 independent runs were averaged. Minimum, mean, and maximum priors for $\Theta$ were 0.0, 20.0, and 40.0 and were 0.0, 25.0, and 50.0 for $M$, respectively.

Tests for the presence of a statistically significant relationship between genetic and geographic distances were carried out using the program IBD (Bohonak 2002). Mantel tests (Mantel 1967) were performed on matrices of pairwise log transformed $D_{EST}$ or $F_{ST}$ and log transformed geographic distances. Geographic distance was measured as the length of coastline between 2 sampling sites by using the path tool in GOOGLE EARTH 5.2.1.1588 (Google Inc., Mountain View, CA).

Results

Microsatellite Diversity

The total number of alleles per locus was moderate, ranging from 8 ($Moe5$) to 28 ($MoeA4$), with a mean number of 17.62 ± 6.59 (Supplementary Table S1; data available on request). Twenty-one private alleles were found distributed among 9 of the 15 sample sites. All these, however, were in very low frequencies (i.e., $\leq 0.6\%$). The location that had the most private alleles was PCA, which also had the largest sample size. Both the total number of alleles and the number of private alleles were significantly correlated with the sample size (Spearman’s rank correlation, $p = 0.68$, $P = 0.005$ and $p = 0.60$, $P = 0.019$, respectively). Mean observed heterozygosity ($H_O$) was moderate to high in all populations, ranging from 0.45 (MH) to 0.75 (CK) and averaged 0.63 ± 0.21 over all populations (Supplementary Table S1).

Exact tests of 117 variable loci by population pairs revealed statistically significant deviations from HWE in only 3 comparisons after Bonferroni adjustment (Rice 1989; $Moe6$ in PI and BH and $Moe12$ in BH; $P \leq 0.05$; Supplementary Table S1). Sixteen of 420 pairwise loci by population tests indicated significant linkage disequilibrium ($P \leq 0.05$); however, none were significant after sequential Bonferroni adjustment, and they were not dominated by any single locus or locus pair.

Population Structure

In general, the smallest values of $D_{EST}$ (and $F_{ST}$) were seen between adjoining sample sites (e.g., TB1–TB3, FB and PCA; Table 2), although this was not always the case. For example, samples sites BS and MH are neighboring sites separated by only ~90 km but their $D_{EST} = 0.54$ (eighth highest of 105), and they do not cluster together in the STRUCTURE analysis (see below; Figures 2 and 3 and Supplementary Figure S1). The Mantel test for spatial autocorrelation indicated a significant ($P \leq 0.002$) but very slight ($r^2 = 0.076$) signal of isolation by distance.

Replicate simulations with STRUCTURE were all very similar indicating that the analysis had converged on the most likely value of $k = 6$. We argue, however, that a more likely value is $k = 7$ (Figures 2 and 3 and Supplementary Figure S1). Even though $k = 6$ has the largest $\Delta k$, the likelihood for $k = 7$ is smaller than $k = 6$ ($-13479$ and $-13654$, respectively), and the primary difference between these 2 scenarios is the disposition of the BT sample. When $k = 6$, individuals from BT are partially assigned to a unique BT group and 2 or 3 of the other groups. The other groups chosen, however, vary across runs. When $k = 7$, most individuals from BT cluster together to the exclusion of all other groups and generally at a higher $Q$ value than when $k = 6$. For those individuals in the BT group with mixed ancestry when $k = 7$, the mixture is predominantly with sample PCA, and this is consistent across simulation runs. For values of $k > 7$, individuals are increasingly partially assigned to multiple groups, and no single sample or group of samples is assigned highly and solely to any of the new groups. Regardless of the value of $k$, groupings of sample sites always include adjacent locations, with the exception of ECS and BS clustering to the exclusion of BT. Most individuals at 3 sites (i.e., PI, LW, and MIB) had highly mixed ancestry values (i.e., low $Q$ values to any single
group; Figure 3 and Supplementary Figure S1) and generally, partially assigned to 2 or more of the defined groups that commonly included the PA, CK, and TB group (Figure 2 and Supplementary Figure S1). None of the 7 groups correspond to the current taxonomy, which also was true when $k = 3$ or 5 corresponding to the number of officially recognized taxonomic units (species only or species and subspecies, respectively).

The MIGRATE run appeared to converge well on the most likely estimates for each parameter, with effective sample sizes large (i.e., mean 1424.74 ± 441.37), posterior distributions unimodally distributed, and the magnitude of the estimated parameter values between the 2 independent runs similar. Consistent with the relatively large pairwise $F_{ST}$ and $D_{EST}$ values, estimates of $N_m$ among sites generally were low (i.e., <1.0; Supplementary Table S2). Fifty one (24.3%) of the 210 $N_m$ estimates were >1.0 (e.g., CK into TB = 2.50), but these corresponded to estimates for sites within the STRUCTURE clusters or those geographically unlikely to actually be exchanging migrants (e.g., MIB and CK). Notably, 49 of these (96.1%) involved either the PA/CK/TB cluster or the sites with individuals of apparent mixed ancestry (i.e., LW and MBI). For the most part, there was no apparent directionality to the migration (Supplementary Table S3).

### Discussion

We used microsatellite loci to evaluate the intra- and interspecific relationships of the *M. corona* species complex. The allelism in this study is comparable to that reported for other gastropods. The 7 microsatellite loci studied from *Littorina saxatilis*, a species with a very similar life history, contained 9–27 alleles per locus (Sokolov et al. 2002) compared with the 8–28 reported here. Not surprisingly, levels of observed heterozygosity also were similar with a mean of 0.63 ± 0.21 and 0.61 ± 0.15 for *M. corona* and *L. saxatilis*, respectively. Although *M. corona* has a fairly narrow species range, it is generally found in large numbers where it occurs. *Littorina saxatilis* has a much broader range occurring in both the northeast and northwest Atlantic and has been observed to occur in extremely high numbers (e.g., 100 000 snails/m²; Reid 1996). The microsatellite heterozygosity seen in *M. corona* is similar to a variety of other gastropods with a wide range of population sizes.
geographic ranges, and levels of population subdivision (e.g., Kelletia kelleti, $H_O = 0.54 \pm 0.26$, White and Toonen 2008; Diloma subrostrata, $H_O = 0.64 \pm 0.17$, Donald et al. 2011; L. litorea, $H_O = 0.48 \pm 0.31$, McInerney et al. 2009). It is likely that in spite of pronounced population subdivision (see below), M. corona populations maintain fairly large genetic effective population sizes and corresponding levels of heterozygosity.

Overall, most pairwise $D_{EST}$ and $F_{ST}$ estimates indicate that populations of crown conchs are not connected by substantial levels of gene flow even over short distances (i.e., tens of km). We think there are several processes shaping the population structure of M. corona, which include habitat continuity, larval and egg dispersal, and both frequent and intermittent genetic exchanges among sites. It must be noted, however, that although we generally think that dispersal is highly restricted for M. corona, rare dispersal events must have occurred over evolutionary time or else M. corona would not occupy the range that it does. The narrowness of the species range likely speaks to the rarity of these events.

Habitat continuity is likely a major factor in the population structuring of M. corona. Along the eastern and Gulf of Mexico seaboards of the United States (New Jersey to Texas) runs, a 4800 km collection of natural inlets, bays, saltwater rivers, and artificial canals known as the Intra-coastal Waterway (ICW). In addition to providing safe navigation among several major US shipping ports, the ICW is an extensive patchwork of marshy habitat for a variety of animals. Long stretches of the ICW are protected and enclosed but punctuated by scattered inlet openings to the Atlantic Ocean or Gulf of Mexico. Water movement in the ICW is primarily due to tidal- and wind-driven circulation with typical tidal currents in excess of 1 m/s at the mouth of an inlet slowing to near zero ~50 km from the inlet (Waterhouse et al. 2011). Clearly, position in the ICW can greatly affect the ability of M. corona to travel from one point to another. For example, both collection sites FB and PCA are within ~50 km of each other with PCA being only ~5 km away from Ponce de León inlet in New Smyrna Beach, FL. Not surprisingly, the $D_{EST}$ and $F_{ST}$ values for these populations are significant but not after Bonferroni adjustment and small (0.028 and 0.011, respectively; Table 2). Comparing either of these to MIB, however, reveals a very different situation. Even though the MIB site is only ~85 km south of the Ponce de León inlet, the average estimates of $D_{EST}$ and $F_{ST}$ between MIB and PCA or FB are statistically significant and large (0.452 and 0.176, respectively). Notably, except for a small (~50 x ~2000 m) artificial canal near Titusville, FL, there is no ICW connection between the MIB and the northern locations. This would mean that for M. corona to move from FB or PCA to MIB, it would have to exit the ICW at Ponce de León inlet and reenter at Sebastian Inlet in Vero Beach, FL (or vice versa). Although overall alongshore transport on that part of the Florida coast is north to south and fairly strong (van Gaalen 2004), this is undoubtedly a rare and much more difficult type of movement compared with that between sites connected by an uninterrupted stretch of the ICW. It is also important to note that, although FB and PCA have likely been connected by the ICW for a long time, artificial canals like the one connecting the MIB and PCA sites are relatively recent (i.e., within the last 100 years). A second illustrative set of sites are ECS, BS, MH, and BT. The ECS and BS sites form a single grouping in STRUCTURE and have small pairwise $F_{ST}$ and $D_{EST}$ values that are not significant after Bonferroni correction (Table 1). They cluster, however, to the exclusion of MH and BT, which are each separate STRUCTURE groups, even though there is less than ~100 km between any of them. The level of divergence between the ECS plus BS group and MH or BT is 0.156–0.365 and 0.307–0.555 for $D_{EST}$ and $F_{ST}$, respectively. Clearly, these groups have experienced very low levels of gene flow. Why then are ECS and BS so similar and not the others? Similar to what was seen with MIB, there are different levels of habitat connectivity among these sites. Except for a couple of small artificial culverts and one artificial channel, the MH site is separated from the southern sites by the land currently supporting Florida State Highway 1 connecting Miami, FL with the Florida Keys. On the other hand, although relatively close to the ECS site (~50 km), BT is in the lower Florida Keys and is separated from the upper keys by an 11 km stretch of open ocean between Marathon, FL and Little Duck Key, FL or 30 km straight-line distance of open Gulf of Mexico water. It is important to note here that M. corona is primarily an estuarine species, and populations are restricted to protected embayments (Leal 2002) and never found in areas exposed directly to the open ocean (Hayes KA, personal observation). It appears that the level of genetic connectivity, therefore, is clearly proportional to the degree of ICW habitat connectivity. On the Gulf of Mexico side of Florida and Alabama, the ICW is less well defined and more open to the Gulf of Mexico and interrupted over large stretches (i.e., the ICW does not extend from Carrabelle, FL to Tarpon Springs, FL, which are just east of our SJP site and just north of TB). Movement of M. corona in North to central parts of Florida (i.e., PA to TB) may not be as influenced by the ICW as the Atlantic coast, which may account for the significant, but smaller $D_{EST}$ and $F_{ST}$ values (0.059–0.108 and 0.015–0.025, respectively), and the clustering indicated by STRUCTURE. The stretch of the ICW connecting OB and the BL plus SJP groups was opened between 1930 and 1934 (Alperin 1983), and the current population subdivision likely reflects the historical separation of these sites.

The life history of M. corona likely also contributes significantly to the population genetic structure indicated by our study. Adult M. corona are relatively heavy and not highly vagile. They produce aplanic lecithotrophic larvae, which likely mediate gene flow among populations. Beginning in late winter and continuing throughout the summer, females lay ribbons of 6–20 egg capsules each housing hundreds of fertilized eggs (Hathaway and Woodburn 1961). Capsules are deposited in the lower intertidal zone on a number of different substrates and have been found on rocks, shells,
wood, polychaete tubes, sea grass, mangroves, bridge pilings, discarded bottles and cans, shoes, and even on the shells of living crown conchs (Clench and Turner 1956; Hathaway 1958; Lof tin 1987). After 20–27 days, fully developed juveniles hatch and may have a brief swimming phase (Lof tin 1987). Commonly, however, juveniles are observed to immediately settle and crawl-away (Gunter and Menzel 1957; Hathaway 1958; Albertson 1980). Clearly, this lifestyle would contribute to highly restricted gene flow among populations. If larvae do experience a brief swimming stage, this would likely facilitate movement among nearby sites connected by contiguous habitat. This can account for the nonsignificant level of population subdivision among the 3 Tampa Bay, FL sites. It may also contribute to the lower levels of subdivision seen in sites connected by uninterrupted ICW as discussed above (e.g., FB and PCA). Given the vagaries of alongshore currents, however, it seems unlikely that the tiny (~1 mm) quickly settling larvae pose a significant avenue for dispersal among sites or sites not connected by the ICW. Concomitantly, estimates of $N_m$ among sites were symmetrical and generally low and for the most part do not indicate evolutionary significant levels of exchange.

Because females are known to deposit their eggs on a variety of mobile substrates, rafting of egg masses and newly settled juveniles also is possible. There are a variety of factors that determine the success and effect of rafting on the evolution of populations. Winds, currents, episodic storms, availability of substrate, entrainment of flotsam in convergence zones, distance among sites, and the ability to feed and reproduce while dispersing all can affect the success of rafting as a means of dispersal (Thiel and Haye 2006). Studies of rafting in populations of the intertidal gastropods Bedea hauyleyi and Barleia spp. indicate that rafting dispersal can rival the potential seen in many planktonic developers (Martel and Chia 1991; Hoskin 2000). Rafting has also been demonstrated to enhance dispersal of mollusks with planktonic larvae when they are associated with buoyant versus non-buoyant substrates (Nikula et al. 2011a, 2011b). Rafting of M. corona egg masses and less so for juveniles, seems to be a plausible mechanism to account for infrequent successful movement among populations. The eggs have a somewhat long development time (up to 27 days) allowing for significant distances to be traversed. The larvae develop inside the egg capsules being nourished by yolk thus mitigating the need for sufficient food to be available during the journey. Unlike the dispersal of single planktonic larvae, the delivery of an egg mass to a site would potentially result in the release of more than 1000 larvae thus increasing the likelihood of persistence of some of the migrants in the new location. This is also an effective method of colonization since the bottleneck effect would be reduced. Avoiding a bottleneck can be further accomplished if there are multiple sires for any single egg mass as has been shown in the related species,Busycon carica (Walker et al. 2007) and in M. corona (Karl SA, unpublished data). Even so, newly established colonies would experience particularly strong genetic drift and could possess dramatically different allele frequencies from the parent population. The viability of rafting of egg masses as a successful dispersal strategy is clearly shown in the “Paradox of Rockall” (Johannesson 1988). The island of Rockall is about 30 m in diameter and lies ~500 km northwest of Ireland, about half way between Ireland and Iceland. Surprisingly, the intertidal zone of Rockall is devoid of invertebrates with planktonic larvae (e.g., barnacles and limpets) but is replete with 17 invertebrates each with nonplanktonic larvae (e.g., the direct-developing L. saxatilis, Johannesson 1988). If M. corona is rafting between sites, the magnitude of genetic divergence indicates that this must be a fairly rare event. Although the species range limit in northeastern Florida is thought to be limited by temperature tolerance (Lof tin 1987), the rarity of rafting and generally North to South direction of alongshore currents in the Gulf of Mexico may be limiting further colonization of the western Gulf of Mexico. The effluent of the Mississippi may also be acting as a barrier (sensu Rocha et al. 2002) pushing dispersers offshore and away from suitable habitat.

Overall, we think that the population structure dynamics of M. corona are an amalgam of frequent short-distance dispersals (i.e., tens of km) via swimming larvae and rafting eggs and juveniles when sites are directly connected by continuous habitat as is typified by the 3 sites in Tampa Bay, FL. Longer dispersal events that may include traversing unsuitable habitat or involving sites separated by land and other disruptions of continuous habitat are much more uncommon and likely only involve rafting eggs. Regardless, M. corona is highly structured and unlikely to experience significant contemporary population exchange.

Historically, 3 species and 3 subspecies of Melongena have been recognized in Florida and Alabama and are collectively referred to as the corona complex. For the most part, these taxonomic distinctions were based on overall size and shell shape, extent of shell spination, and shell color. Not surprisingly, the taxonomy of these groups has changed several times. A previous molecular study (Hayes and Karl 2009) surveyed genetic variation in mitochondrial DNA of all extant members of the genus Melongena including the corona complex. Although some of the existing taxonomy was supported (e.g., the monophyly of the genus Melongena), there was neither support for the subspecies status in M. corona nor for species status of M. sprucecreckensis and M. bicolor, and Hayes and Karl (2009) recommended that all be considered M. corona. Our nuclear-encoded microsatellite data echo this conclusion. Although there were clear and sometime strong genetic differences between many of the sample sites, none of these distinctions corresponded to the current taxonomy. In particular, if individuals in the Spruce Creek Estuary, FL. are to be considered a separate species (i.e., M. sprucecreckensis), then the level of genetic divergence between the PCA and FB sites (i.e., M. bicolor) is such that individuals at the FB site should also carry this binomial. Similarly, if the individuals at the BS site are to be considered M. c. altispira so should those at the ECS site
(M. bicolor). The levels of genetic divergence between all the sites are significant and sometimes very large (e.g., MH vs. OB; $D_{EST} = 0.731$ and $F_{ST} = 0.438$), but the level of divergence seen within recognized taxa of the corona complex is equal to or larger than that seen between taxa. Given the magnitude of sharing of mitochondrial DNA haplotypes previously reported (Hayes and Karl 2009), the lack of concordance with existing taxonomy, and the lack of convincing morphological differentiation, we think that M. corona complex should be considered a single, albeit highly structured, species.

Supplementary Material
Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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