POPULATION GENETIC STRUCTURE OF THE TEMPERATE SCLERACTINIAN CORAL, *OCULINA ARBUSCULA*, IN COASTAL GEORGIA

by

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(Under the Direction of Daniel F. Gleason)

ABSTRACT

Dispersal of marine organisms has generally been assumed to be demographically open with planktonic larvae exploiting ocean currents to travel long distances. However, recent studies report evidence of local larval recruitment. In this study, I hypothesized that populations of the temperate coral, *Oculina arbuscula*, are maintained primarily by local recruitment off the Georgia coast because: 1) suitable substrate for settlement is patchy and 2) *O. arbuscula*'s branching morphology is conducive to asexual reproduction by fragmentation. To address this hypothesis, I combined data from allozyme electrophoresis, histocompatibility assays, and dispersion of established colonies. Results indicated substantial local recruitment maintained primarily by sexual reproduction rather than asexual fragmentation. Sexually produced planulae are not only philopatric, but also show evidence of some migration among populations. In this temperate species, local recruitment primarily maintains aggregated populations of *O. arbuscula*.

INDEX WORDS: *Oculina arbuscula*, Corals, Recruitment, Larval dispersal, Allozyme Electrophoresis, Histocompatibility, Grays Reef

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BA, Ohio Wesleyan University, 2002

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

STATESBORO, GEORGIA

2006

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April 2006

DEDICATION

I dedicate this work to my parents, Gary and Debbie, and my sister, Jacqueline. No words can describe how grateful I am to have their unconditional love, support, and guidance in all I set out to do. Without them, I would not be where I am today. I also dedicate this thesis to my dogs, Chase and Halle. No matter how bad things are, they always make me smile.

ACKNOWLEDGMENTS

I wish to thank my major professor, Dr. Daniel F. Gleason, for his guidance and financial support throughout this project. I am also grateful to the other members of my graduate review committee, Dr. Alan Harvey and Dr. Michelle Zjhra, who took time to criticize many revisions of my work, offering their constructive comments and valuable suggestions.

The field work was made possible with the help of many people. I extend special thanks to my dive buddies, Rob Ruzicka, Chris Freeman, Leslie Bates, and Sarah Mock, whose help was invaluable both under the water and back on the rocking waves of the surface. Thank you to all employees of Gray's Reef National Marine Sanctuary, with a very special thanks to Greg McFall, coordinator of research at the sanctuary. I am also indebted to the captains and crew of the NOAA ship Nancy Foster for their hard work.

I would also like to thank Dr. Daniel Brazeau, University of Buffalo, who provided me with much of my initial research equipment. His seasoned guidance made the electrophoresis experiment less formidable an endeavor. Data analysis help was provided by Dr. D. Kelly McLain (GSU), Dr. William Irby (GSU), Dr. C. Ray Chandler (GSU) and Carla Zilberberg (Universidade Federal do Rio de Janeiro). The computer program used to calculate expected genotypic diversity (G_E) was developed by Zohar Pasternak. Thank you also to Dr. Sandra Brooke for providing Florida coral samples.

This research was partially funded by grants from Gray's Reef National Marine Sanctuary (part of NOAA's National Marine Sanctuaries program) and the Georgia Southern University Graduate Student Professional Development Fund Research and Travel Grants.

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LITERATURE REVIEW

Dispersal is important for the spread and survival of species, so determining whether geographically separate populations are genetically distinct is a key ecological question. Populations stay connected genetically through the process of gene flow via dispersal. Dispersal is the passive or active movement of individuals between populations. Populations that are not connected by gene flow may diverge over time or undergo speciation due to processes such as genetic drift or local adaptation (Hartl and Clark 1997). In general, large, mobile organisms face fewer barriers to dispersal than small, sessile organisms (Cain et al. 2000, Stevenson and Adams 2000). The latter have no or limited mobility as adults and have evolved alternate ways to disperse, such as hitching a ride on mobile organisms, traveling by wind, or using water currents (Dorit et al. 1991). A strategy for long distance dispersal in small, sessile organisms is the mass release of seeds or young to compensate for massive mortality resulting from predation, starvation, or inability to find suitable substrate for settlement (Pechenik 1999).

Dispersal of marine organisms has generally been assumed to be demographically open with planktonic larvae exploiting ocean currents to travel long distances (Roberts 1997). However, recent studies report evidence of local larval recruitment (Cowen et al. 2000, Stevenson and Adams 2000, Mora and Sale 2002, Cowen et al. 2006). For example, in Australia a higher proportion of fish larvae were found returning to their natal reef than was predicted by chance alone (Jones et al. 1999, Thorrold et al. 2002) and allozyme studies of various brooding and broadcast spawning coral species also found local recruitment (Ayre and Hughes 2000, Whitaker 2004).

Recruitment is the successful establishment of new individuals in an environment to live/reproduce as part of that population and local recruitment is the establishment of individuals in their natal habitat. Local recruitment can be advantageous in marine systems to avoid large losses of planktonic larvae through predation or to ensure larvae contact suitable substrate (Strathmann 1985, Perrin and Mazalov 2000). Larvae that disperse long distances are not guaranteed to find hospitable settlement sites, especially in environments where suitable habitat is fragmented or patchy (Cook et al. 2002).

Marine species exhibit a range of reproductive and dispersal strategies. For example, scleractinian corals can reproduce sexually by broadcasting gametes (Shlesinger et al. 1998) or by releasing fully developed brooded larvae into the water column (Ayre and Resing 1986, Fukami et al. 2003). Larvae produced from broadcasted gametes typically have longer competency periods than those that are brooded; however, broadcasted larvae have exhibited rapid settlement in some species (Fadlallah 1983, Miller and Mundy 2003).

Coral larvae (i.e., planulae) metamorphose into a single stationary polyp after settlement and secrete a calcium carbonate skeleton, created with energy from symbiotic zooxanthellae (Muscatine and Porter 1977) and ingestion of food particles by the polyp (Piniak 2002). Coral polyps in the same skeleton are all clones of the same genotype (genet) (Hughes and Jackson 1985). These skeletons can sometimes break or fragment (Highsmith 1982), creating a new individual with the same genotype as the parent colony (ramet). Due to asexual reproduction, it is possible to have hundreds of colonies on a reef composed of only a few genotypes (Hughes and Jackson 1985). Asexual reproduction by

fragmentation leads to local recruitment, while spawned larvae may disperse longer distances (Fadlallah 1983, Jackson 1986).

Dispersal and recruitment patterns in corals, as well as other marine species, have generally been inferred through a combination of molecular techniques and field surveys of existing populations. In the field, transect and plot surveys help decipher the effect of recruitment on adult distributions by examining dispersion patterns within populations (Carlon and Olson 1993). More aggregated populations point towards larvae settling close to parents, populations consisting of predominantly clonal individuals, larvae using specific settlement cues, or differential post-settlement mortality. Allorecognition occurs in many clonal marine species allowing use of histocompatibility assays to determine dispersal distances between related individuals (Neigel and Avise 1983, Grosberg and Quinn 1986, Grosberg 1988, Hughes et al. 2004). If individuals within the same population exhibit histocompatibility, then local recruitment occurs, but a panmictic species may exhibit histocompatiblity among all populations.

Genetic techniques can also help determine whether marine populations are demographically linked or isolated (Ouborg et al. 1999, Ayre and Hughes 2000, Ng and Morton 2003). Allozyme electrophoresis is commonly used in population genetic studies of corals because other molecular techniques are difficult to use on these organisms (Brazeau and Harvell 1994, Burnett et al. 1994, Adjeroud and Tsuchyia 1999, Yu et al. 1999, Ayre and Hughes 2000, Ng and Morton 2003, Whitaker 2004). Microsatellite primers and mtDNA markers have proved difficult to develop, costly, and time consuming in many species of corals (Marquez et al. 2003, Ayre and Hughes 2004, Brazeau et al. 2005). Fingerprinting techniques (such as AFLP's) have been successful

in determining the source of coral spat when assaying several adult populations, but were not appropriate for this study because I was unable to identify newly settled recruits and was interested in comparing allele frequencies among several populations (Brazeau et al. 2005). Thus, I selected allozyme electrophoresis for my genetic assays. There are some drawbacks to using allozymes, such as: 1) silent mutations of the genetic code are not detectable, 2) migration rates of alleles do not indicate relationships between them, and 3) banding patterns are sometimes difficult to interpret (Burton 1994). Despite these shortcomings, allozymes are a relatively inexpensive and reliable technique for assessing gene flow among populations and for identifying the contribution of asexual reproduction to population maintenance (Stoddart 1983b, Burton 1994).

The number of polymorphic loci used in allozyme studies of corals has ranged from 4 to 8 with a mean of 5.69 (n = 12, SD = ± 1.49) (Brazeau and Harvell 1994, Adjeroud and Tsuchyia 1999, Yu et al. 1999, Ayre and Hughes 2000, Dai et al. 2000, Ng and Morton 2003). In my study, six polymorphic loci and two monomorphic loci were found in the Georgia *Oculina arbuscula* populations, falling within the published range. Sample sizes also vary in past coral studies from 18 to 70 individuals per population. It is assumed that larger sample sizes are better (Kalinowski 2005), but it is more a combination of sample size and the number of polymorphic loci. With fewer polymorphic loci, a larger sample size is necessary. This study used six polymorphic loci and at least 29 samples per population which again fits with previously published works of five loci and 19 samples in *Briarium asbestinum*, seven loci and 18 samples in *Mycedium elephantotus*, and seven loci and 30 samples in *Platygyra sinensis* (Brazeau and Harvell 1994, Yu et al. 1999, Ng and Morton 2003).

The primary objective of the present study was to determine whether populations of the temperate coral *O. arbuscula* use local or long distance dispersal to maintain populations off the coast of Georgia. This study combined the previously described field and molecular techniques to determine dispersal distance. *Oculina arbuscula* is a branching, scleractinian coral found off the coast of North Carolina, Georgia, and north Florida. The reproductive biology of *O. arbuscula* has not yet been studied, but reproductive traits are known for a neighboring related species off the coast of Florida, *Oculina varicosa* (Brooke and Young 2003). Coral species within the same genus and geographic region are likely to have similar modes of reproduction (85% of genera contain species with the same reproductive mode, out of 41 genera in 7 regions) (Richmond and Hunter 1990). Therefore, the reproductive characteristics of *O. arbuscula* are thought to be similar to those of its congener, *O. varicosa*. The gametes of *O. varicosa* are broadcast, but the eggs have been shown to be negatively buoyant (Brooke and Young 2003). The planulae are initially negatively geotaxic and can stay in the water column up to 21 days, potentially dispersing long distances on ocean currents. *Oculina arbuscula* can also reproduce clonally by fragmentation, due to its branching morphology. Therefore, a secondary objective was to determine whether *O. arbuscula* reproduces more by asexual fragmentation or sexually derived larvae.

INTRODUCTION

Understanding recruitment processes in marine populations is important in an ecological context and has relevance to both commercial and conservation issues. It has been assumed traditionally that marine populations are demographically open with new individuals coming from planktonic larvae that disperse over long distances in the water column (Roberts 1997). More recent studies have challenged this idea by showing evidence of more local dispersal that results in genetically isolated populations at the regional scale (Cowen et al. 2000, Cowen et al. 2006). Long-distance dispersal should not be completely discounted. Longer dispersal distances may be necessary to allow gene flow between populations, to allow invasion of new habitats, and to avoid competition with relatives (Jackson 1986, Pechenik 1999). Local retention of propagules also has advantages, such as minimizing predation risks through a reduced planktonic period and decreasing mortality resulting from settlement in an unsuitable habitat (Vance 1973, Bullard et al. 1999).

The distance marine larvae disperse is influenced by both biotic and abiotic factors. The three biological components are: 1) duration of the planktonic life, 2) larval swimming behavior, and 3) timing of larval/gamete spawn (Scheltema 1986, Ellien et al. 2004). Physical components, such as speed and direction of transport currents and substrate availability, also influence dispersal distances (Scheltema 1986, Cowen et al. 2006). Few studies have been able to incorporate all of these components when studying or modeling marine planktonic dispersal, and the disproportionate weight placed on larval duration inevitably led to the conclusion that species with long planktonic periods travel long distances (Scheltema 1986, Caley et al. 1996). However, a long larval stage does

not necessarily lead to broader geographic dispersal (Bhaud 1998). A recent model using all of the physical and biological factors listed above found typical larval dispersal distances in fish to be only 10-100 km (Cowen et al. 2006). Other recent studies on fish and invertebrates support this view of shorter dispersal distances (Swearer et al. 1999, Barnay et al. 2003, Ayre and Hughes 2004, Cowen et al. 2006).

Reef-building corals are a diverse group of benthic cnidarians with various reproductive modes and dispersal methods. Corals can broadcast gametes, brood larvae, or reproduce asexually by budding, fragmentation, or polyp expulsion (Highsmith 1982, Fadlallah 1983, Stoddart 1983a, Ayre and Resing 1986, Shlesinger et al. 1998, Adjeroud and Tsuchyia 1999). The general consensus is that larvae produced by broadcasters disperse longer distances than propagules resulting from brooding or asexual reproduction (Fadlallah 1983, Ayre et al. 1997, Altieri 2003, Nishikawa et al. 2003). However, a recent study that observed short competency times and rapid settlement in two broadcast-spawning coral species suggests that broadcasting gametes may contribute more to local recruitment than previously thought (Miller and Mundy 2003).

The temperate coral *Oculina arbuscula* is found off the coast of North Carolina (Miller 1995) and Georgia (Fioravanti-Score 1998) and is the only branching scleractinian coral found on reefs off the Georgia coast. The reproductive characteristics of *O. arbuscula* have not been described, but this information is known for a related species, *Oculina varicosa*, found off the Florida coast (Brooke and Young 2003). These species are closely related and may even overlap geographically in North Florida, with no known hybridization (Avise 2000). Coral species within the same genus and geographic region are likely to have similar modes of reproduction (85% of genera contain species

with the same reproductive mode, out of 41 genera in 7 regions) (Richmond and Hunter 1990). Therefore, the reproductive traits of *O. varicosa* should be similar to *O. arbuscula* and attributable to this study. *Oculina varicosa* is a gonochoristic, broadcast-spawning species (Brooke and Young 2003). Its eggs are negatively buoyant, the planulae exhibit negative geotaxis, and the planktonic larval duration is up to 21 days, with benthic-probing behavior often exhibited after 1-2 weeks (Brooke and Young 2003). The branching morphology of *O. arbuscula* also increases the probability that it will reproduce asexually via fragmentation, with the resulting clone staying close to the adult from which it originated. Survival of fragments in tropical species of branching scleractinian corals are size dependent with an overall survival rate of 39% (Highsmith et al. 1980). Reproduction via fragmentation is so common and successful in branching corals that many studies find mostly local recruitment of fragments with just enough sexual reproduction to maintain genetic variability within populations (Kojis and Quinn 1981b, Adjeroud and Tsuchyia 1999, Ayre and Hughes 2000).

This study aimed to determine dispersal distances of the temperate coral, *O. arbuscula*, off the coast of Georgia, U.S.A. I hypothesized that *O. arbuscula* populations are maintained primarily by local recruitment of coral fragments due to the branching morphology of *O. arbuscula* and the patchy distribution of hard-bottom substrate needed for settlement. I addressed this hypothesis by combining data from three sources: 1) belt transects to document the distribution of adult colonies, 2) histocompatibility assays to investigate genetic relatedness between and within populations, and 3) allozyme electrophoresis to assess population genetic structure. The many possible outcomes and explanations for potential results are displayed as a flow chart in Figure 1.

MATERIALS AND METHODS

Study Sites

This study was conducted with corals collected from 5 live bottom reefs found in and around Gray's Reef National Marine Sanctuary (GRNMS, Figure 2). GRNMS is located 34.2 km east of Sapelo Island, GA. Two sites were within the GRNMS boundaries, the Gray's Reef Monitoring Site (**MS**, 31º 23.815 N, 80º 53.461 W) and Patch Reef #1 (**P1,** 31° 24.340 N, 80° 51.983 W). The other three reefs lied outside GRNMS and included: J Reef (**JR,** 31º 36.056 N, 80º 47.431 W), the R2 Tower hard bottom (**R2,** 31º 24.305 N, 80º 35.490 W), and Ledge #6 (**L6,** 31º 37.688 N, 80º 34.662 W). Pairs of reefs were separated by 1.8 to 39 km (Figure 2).

The reefs sampled in this study ranged in depth from 20-30 m and varied in extent and substrate composition. Three of the reefs (MS, JR, and L6) are structured as ledge systems with vertical relief hard-bottom leveling off into a sandy plateau habitat (Table 1). The 130 m long ledge composing the Gray's Reef Monitoring Site consists of aragonitic limestone with vertical ledge relief between 0.5 to 1 m (Hunt 1974). J-Reef is a ledge constructed of relic scallop shells with a maximum relief of 1 m. The substrate at Ledge #6 is aragonite with a maximum height of 1.5 m. All three of these ledges are oriented north/north-east to south/south-west.

The other two Georgia reef communities sampled are built on clumps of rock protruding from the sandy bottom, as opposed to the previously described ledge systems. Gray's Reef Patch #1 consists of large aragonite rock slabs jutting up from the sand. The entire patch reef is only about 30 x 13 m, but this reef has the highest vertical relief ranging from 0.5 to 2 m off the sand. R2 Tower live bottom is a series of small (0.5 – 2

m diameter) aragonitic rocks separated by sand and emerging no more than 0.5 m above the substrate.

Adult Distributional Patterns

I used belt transects to determine if established colonies of *O. arbuscula* are distributed in a uniform, random, or clumped fashion (Carlon and Olson 1993). I ran five 1 x 10 m belt transects at the five Georgia reefs (MS, JR, L6, P1, R2). The position of every *O. arbuscula* colony found within these belt transects was documented by recording the location of the colony along the line as well as the distance from the line to the colony (up to 50 cm distant on either side of the line) in a perpendicular direction. To determine if the corals were distributed in a clumped or random pattern, belt transect data were assessed for deviation from a Poisson distribution. To run the Poisson test I needed sufficient replication and a sufficient number plots with no corals (Sokal and Rohlf 2001), so the belt transects were divided every meter resulting in fifty 1 x 1 m plots with 0 to 11 corals per plot.

A coefficient of dispersion was calculated from the plot data using the statistic $I = (s^2 / X)(n-1)$, where s = standard deviation, X = the mean, and n = sample size (Sokal and Rohlf 2001). The resulting 'I' value was compared to a χ^2 distribution with (n – 1) degrees of freedom. Values less than or exceeding the critical χ^2 values ($\chi^2_{(0.025)(df)}$ and $\chi^2_{(0.975)(df)}$) indicated a uniform or aggregated dispersion pattern respectively. Values that fell between the critical values indicated a random distribution.

Histocompatibility Assays

Histocompatibility assays based on allorecognition between individuals can be used in many clonal marine species to estimate relatedness (Neigel and Avise 1983,

Grosberg and Quinn 1986, Grosberg 1988, Hughes et al. 2004). Individuals that have similar genetic composition (self or kin recognition) will exhibit fusion of tissue over time when put in physical contact whereas genetically distant individuals will show a rejection response (Chornesky 1989, Feldgarden and Yund 1992). A subset of the *O. arbuscula* populations were used in a histocompatibility assay to assess relatedness within and among the five Georgia populations. Concrete pavers (15 x 15 cm) were placed on the J-Reef ledge during May 2004. Two corals were attached to each paver using underwater epoxy and positioned so that there was contact between the branch tips where rapid growth occurs. There were 3 treatment groups (i.e., contact between two individuals from different populations) and 3 control groups (i.e., contact between two individuals from the same population) in this experiment.

In June 2004, populations MS, JR, and L6 were used to determine if there was more compatibility within these populations than between pairings of different populations. Treatments consisted of the following pairings: MS-JR, MS-L6, and L6-JR. Likewise, controls were as follows: MS-MS, JR-JR, and L6-L6. Out of 8-12 replicates for each treatment and control at the beginning of the study, as few as 2 remained at the time of data collection with 41 total survivors (Table 2). In October, I used a plastic magnifying lens to observe the experimental corals in situ and determine whether they were compatible. Corals that were not compatible exhibited necrosis, a dark band of tissue at the point of contact, whether or not the skeleton fused (Figure 3). Compatible corals had fusion of skeleton and tissue, with no obvious tissue necrosis. Corals with no tissue fusion and no necrosis of tissue were scored as incompatible.

The study was repeated in summer 2005 with different populations. In May, corals were deposited on 88 pavers from the three southern latitude reefs (P1, R2, and MS). The design was similar to 2004, with treatments consisting of across reef pairings (MS-P1, MS-R2, and R2-P1) and controls consisting of within reef pairings (MS-MS, P1-P1, and R2-R2). Three weeks after initial set-up, I went back to epoxy any corals that were not well secured. Out of 12-15 replicates for the treatments and controls, 9-15 remained at the time of data collection and 75 total survived (Table 2). In October, the same method used the previous summer was incorporated to observe the corals and determine compatibility. I used a χ^2 test to determine if corals from within the same population fused at a higher rate than corals from different populations for the 2004 data and 2005 data.

Population Genetic Structure

Collection of *O. arbuscula* for electrophoresis occurred during June - August 2003 and March 2004. Fragments (3-5 cm long) of 29-35 *O. arbuscula* colonies were collected haphazardly from the five Georgia reefs (Figure 2). The samples were snap frozen in liquid nitrogen to be transported from the field site to the laboratory and transferred to an ultracold freezer (-70º C) where they were stored until removed for further analysis.

Cellulose acetate allozyme electrophoresis was used to determine the population genetic structure of *O. arbuscula* off the Georgia coast. Of the 18 allozymes tested in *O. arbuscula* (Appendix A), 6 were found to be polymorphic and produced repeatable bands. These 6 allozymes were phosphoglucose isomerase [*PGI*: Enzyme Commission Number (EC) 5.3.1.9], malate dehydrogenase (*MDH*: EC 1.1.1.37), phosphoglucomutase

(*PGM*: EC 5.4.2.2), mannose-6-phosphate isomerase (*MPI*: EC 5.3.1.8), creatine kinase (*CK*: EC 2.7.3.2) and aldehyde dehyrogenase (*AD*: EC 1.2.1.3).

Samples of *O. arbuscula* were removed from the freezer and a small piece (approx. 5 mm long) was broken off the end of the coral branch. Tissue homogenates were prepared by grinding coral fragments individually with 1 mL of grinding buffer (0.05 M tris-HCl buffer, pH 8) using a mortar and pestle (Hebert and Beaton 1993). This mixture was then placed into a 1.5 ml microcentrifuge tube and centrifuged at 1000 rpm for 10 minutes. The tissue homogenate was centrifuged to reduce contamination by skeleton, mucous, and zooxanthellae, though previous genetic work with hermatypic corals established that enzymes of the coral's symbiotic zooxanthellae do not confound the protein genetic work of the coral itself (Ayre and Resing 1986). I also tested this assumption by running centrifuged and un-centrifuged samples on a gel. This test resulted in similar banding patterns for both samples.

Before running the gel, I loaded each of the buffered solutions onto the cellulose acetate gel and placed the gel into the electrophoresis rig (Hebert and Beaton 1993, Kolodziejczyk et al. 2002, Brazeau pers. comm.). The gel ran at 190-200 volts for 20- 30 minutes (Hebert and Beaton 1993, Kolodziejczyk et al. 2002). The gel was removed from the gel box, blotted dry, and placed onto the staining plate. Molten agar at 60º C combined with the necessary staining chemicals was poured onto the gel, revealing the specific protein migration bands. Electrophoretic buffers and enzyme stain recipes followed those of Hebert and Beaton (1993). The homozygosity and heterozygosity of each allele was evaluated by measuring the migration of individual protein bands on the gels. Zymograms were measured in mm from the origin of migration to the darkest part

of the band. I confirmed accuracy of these measurements by blindly re-measuring at a later time and comparing the results (of 440 duplicate measures, 92% matched the original measurement). The most common allele in each of the six polymorphic enzymes was assigned a mobility of *100*, with faster alleles given a higher value (i.e. *105*-*138*) and slower alleles a lower value (i.e. *94*-*84*).

Numerous measures of genetic variability were included in this study. The magnitude and direction of departure from Hardy-Weinberg equilibrium were calculated as D_S values for each population (D_S = [Observed Heterozygosity – Expected Heterozygosity] / Expected Heterozygosity). Negative D_S values indicated population variations due to deficits of heterozygotes and positive values indicated excess heterozygotes (Selander 1970). Frequencies of observed and expected heterozygosity were assessed with a single-classification test for goodness of fit (G) and adjusted for small sample size using a William's correction (Sokal and Rohlf 2001). Tests for linkage disequilibrium were done using the program FSTAT Version 2.9.3.2 (Goudet 2001). To test whether each locus behaved independently, a contingency table for all pairs of loci in each population was constructed (probability was based on 90,000 permutations). Significant values may result from either physical linkage of the loci (indicated by similar patterns of linkage within each population) or inbreeding, random drift, mutation, selection, or asexual reproduction. Populations that are reproducing asexually may be, by chance, in single-locus Hardy-Weinberg equilibria, but will not be in multi-locus linkage equilibria.

To test whether populations of *O. arbuscula* are reproducing sexually or asexually, clonal diversity and genotypic diversity values were calculated. The number

of unique multilocus genotypes (N_C) divided by the total number of individuals sampled from the population (N_I) resulted in a clonal diversity value. Values less than one resulted from duplicates of genotypes in the population. The ratio of genotypic diversity observed (G_O) to genotypic diversity expected (G_E) was calculated as described by Stoddart (1983b) to estimate the contribution of asexual reproduction to current population structure. Low multilocus genotypic diversity values were indicative of departures from equilibria attributable to asexual reproduction.

Nei's genetic distance and F-statistics were used to quantify genetic variation and gene flow between populations. Nei's genetic distance calculated genetic similarity or difference between populations (Nei 1978). POPGENE Version 1.31 (Yeh et al. 1997) generated these calculations and also created an UPGMA dendrogram (unweighted pair-group method using arithmetic averages). F-statistics indicate a loss of heterozygosity between populations (F_{ST}) due to genetic drift or within populations (F_{IS}) due to inbreeding (Wright 1978). FSTAT 2.9.3.2 calculated the F_{ST} values between every pair of populations. Significance was calculated using a bootstrap technique for 95% confidence intervals. A jackknifing procedure calculated standard errors of F_{ST} over all loci (Weir and Cockerham 1984). F_{ST} and F_{IS} values were also calculated for all populations at each locus and overall using POPGENE 1.31. To estimate gene flow between populations, I calculated effective number of migrants in each population (N_m) from the F_{ST} values using the formula $(1/F_{ST} - 1)/4$. N_m values < 1 indicate that genetic drift has been occurring over time, while values > 4 indicate that gene flow has occurred between populations (Avise 2000).

RESULTS

Adult Distributional Patterns

Established *O. arbuscula* colonies were distributed in a clumped fashion at MS, P1, R2, and JR, while corals at L6 were distributed randomly (Figure 4). Reefs at MS and P1 had the highest colony densities followed by JR (Figure 5). Colony densities were lowest at R2 and L6, with many plots lacking corals altogether.

Histocompatibility Assays

In 2004, *O. arbuscula* colonies exhibited some compatibility within as well as between reefs (Table 2). Corals from the most distantly related population, L6, were not compatible with corals from the other two populations, JR or MS. However, due to small sample sizes, there was no significant difference in compatibility for corals paired within versus between reefs ($\chi^2 = 2.38$, df = 2, p = 0.30).

Even though strengthened by a larger sample size (41 total in 2004 vs. 75 total in 2005), no differences in histocompatibility within and between reefs were observed in 2005 (Table 2, $\chi^2 = 2.46$, df = 2, p = 0.29). This result occurred even though all three reef populations used in 2005 were more closely related (as determined through allozyme data) than the genetically distant L6 population used in the 2004 study. Surprisingly, although not significant, the number of fusions was higher for colonies paired from different reefs than within reefs.

Population Genetic Structure

I assayed 18 enzymes with 6 (*GPI*, *PGM*, *MPI*, *MDH*, *CK*, *AD*) exhibiting polymorphism at a single locus (Table 3). Two other enzymes *FUM* and *HK*, exhibited a

monomorphic banding pattern for *O. arbuscula*, while the other 10 enzymes did not develop bands on a gel (Appendix A).

Oculina arbuscula populations at the northern latitude reefs (JR and L6) tended to be more similar to each other genetically than to the southern latitude reefs (MS, P1, and R2). There were two alleles shared among the southern latitude populations, *CK 138* and *PGM 105*, that were missing from the two northern latitude populations (Table 3). There was no significant difference among the five populations in terms of the number of alleles per locus (ANOVA = 0.495, df = 4,26, p = 0.74), but the tendency for the populations to be structured along latitudinal lines can be seen in Nei's genetic distance (Figure 6) and in F_{ST} calculations (Table 4).

The observed frequency of heterozygosity in each population was significantly lower than expected which resulted in negative D_S values and all populations being out of Hardy-Weinberg equilibrium (Table 5). Departures from equilibrium usually coincide with asexual reproduction in populations of clonal animals, but may also be an indicator of population subdivision. Linkage disequilibrium did not explain deviations from Hardy-Weinberg because only one pair of loci in the JR population was found to be significantly linked. All other pairs of loci in the populations were not linked (Appendix B).

Values for Nei's unbiased genetic distance (D) ranged from 0.047- 0.150, smaller values indicated more closely related populations (Table 4). The two most closely related populations of *O. arbuscula* were P1 and R2. The least related Georgia populations were MS to L6 and P1 to L6. In the UPGMA cluster dendrogram the three southern

populations (MS, P1, and R2) formed one cluster and the two northern populations (JR and L6) another (Figure 6).

Pairwise comparisons of allele frequencies among the five Georgia populations using Wright's F-statistics indicated significant differences in all cases except between P1 and R2 (Table 4). A visual representation of the amount of gene flow between reefs and their geographic proximity is given in Fig. 7. When F_{ST} values for all populations are included, there is a trend for more geographically distant populations to be less similar genetically than those in closer proximity (Figure 8). Removing the outlier point with a bivariate normal ellipse (Sokal and Rohlf 2001) and re-running the regression analysis reveals an even stronger positive relationship between F_{ST} values and the distance between reefs. Based on the F_{ST} values, the number of effective migrants per generation between populations was highest between P1 and R2, followed by P1 and MS (Table 4). F_{ST} and F_{IS} values were significant across all loci in all of the populations indicating a deficit of heterozygotes both among and within populations (Table 6).

The contribution of asexual reproduction to the maintenance of these coral populations was assessed using clonal diversity (N_C:N_I) and genotypic diversity (G_O:G_E) measures (Table 7). In a sexually reproducing population in panmixia, the expected result for the G_O:G_E ratio would be around 1.0. The results for *O. arbuscula* were between 0.66 and 1.05 with a mean of 0.88 for the five Georgia populations studied. These results indicate populations of *O. arbuscula* are maintained primarily by sexual rather than asexual reproduction. The N_C:N_I ratios also indicated that more than half of the individuals had unique genotypes in the *O. arbuscula* populations, providing

additional support that *O. arbuscula* on Georgia reefs are maintained primarily by sexually produced recruits.

DISCUSSION

Dispersal distances may not only be determined by the biology of the organism, but also by the environment in which the organism resides. An organism in a large, stable habitat may disperse long distances if suitable settlement sites are common, whereas an organism in a patchy, fragmented habitat may benefit more from local retention of offspring (Robinson et al. 1992, Cook et al. 2002). Planktonic larvae of marine species have traditionally been viewed as dispersing long distances; however, there has been greater recognition of the prevalence of local recruitment as knowledge of larval behavior and physical oceanic processes has increased (Cowen et al. 2000, Cowen et al. 2006). Studies of planktonic dispersal in cnidarians tend to focus on tropical species and continuous reef systems where both local and long distance dispersal have been observed (Burnett et al. 1994, Ayre and Hughes 2000, Bastidas et al. 2001). My study shifted focus to a temperate reef system off Georgia where suitable hard-bottom habitat is patchily distributed and not readily available.

Three assays were used to determine the primary dispersal patterns and reproductive modes for Georgia *O. arbuscula* populations. Results of allozyme electrophoresis and coral dispersion surveys on five *O. arbuscula* populations are supportive of a local recruitment hypothesis with varying contributions from asexual reproduction. In contrast, histocompatibility results were inconclusive. Thus, despite the capability for long distance planktonic dispersal, *O. arbuscula* populations appear to be recruiting locally; a strategy that may ensure survival of offspring in a patchy temperate reef environment.

Local recruitment is becoming a common theme in marine populations with sessile adults and pelagic larvae. Theories on dispersal would assume these populations utilize their pelagic larvae for long distance dispersal to invade new habitats and spread genes between populations. However, significant genetic differentiation between populations has been found in soft corals (Bastidas et al. 2001), scleractinian corals (Whitaker 2004), zooanthids (Burnett et al. 1995), and clonal ascidians (Ayre et al. 1997). Although *O. arbuscula* has a high dispersal potential, it too showed significant genetic differentiation and limited gene flow between reef populations. Incomplete mixing of larvae and genetic subdivision are becoming increasingly common in coral population genetic studies and have been observed in a diverse array of scleractinians including *Acroporids* in western Australia (Whitaker 2004), *Pocillopora damicornis* in Japan (Adjeroud and Tsuchyia 1999), and *Mycedium elephantotus* in Taiwan (Yu et al. 1999).

An inverse correlation between gene flow (N_m) and distance is expected in populations of sessile marine invertebrates. This is the case for *M. elephantotus*, where gene flow for regions > 80 km apart ranged between 0.90 to 7.56 effective migrants per generation, but was much higher (N_m = 11.11 – 24.75) between populations within regions separated by < 20 km (Yu et al. 1999). *Oculina arbuscula* populations also showed an effect of distance on gene flow, the most distant populations (MS to L6 = 39 km) had the least migration (N_m = 4.21) and the closest populations (MS to P1 = 1.8 km) had more migration (N_m = 10.17). Substrate availability was not researched for this study, but could influence the gene flow between *O. arbuscula* populations. The substrate is erratically distributed off the Georgia coast as a series of ledges and patch reefs (Hunt 1974). Five large representative reefs were used in this study; however, it is

unknown how many hard-bottom habitats exist between these five study sites. There are possibly thousands of small suitable habitats between study reefs that may act as stepping stones for dispersal. Similar to stepping stone models in terrestrial systems, increasing the amount of available substrate in a marine environment could better the chance of recruit survival and contribute to higher overall gene flow between populations (Kronforst and Fleming 2001).

Restricted dispersal of gametes and larvae could explain the large deficit of heterozygotes across sets of unlinked loci in Georgia *O. arbuscula* populations. Heterozygote deficiencies resulted in populations of *O. arbuscula* being out of Hardy-Weinberg equilibrium. Heterozygote deficiencies are common in scleractinian coral populations (Yu et al. 1999, Whitaker 2004). In clonal organisms, reductions in heterozygotes are often attributed to asexual reproduction; however, my data shows that sexual reproduction occurs frequently in *O. arbuscula* populations. Deficits in heterozygotes may also be caused by disproportionately higher mortality of heterozygous individuals, reduced fitness of heterozygotes, or spawning of homozygous individuals at different times (Miller and Benzie 1997).

Arborescent clonal marine invertebrates, like *O. arbuscula*, can reproduce via sexually produced propagules or asexually by fragmentation. My results show that *O. arbuscula* populations are maintained primarily by sexual reproduction, as evidenced by the high genotypic diversity (G_O:G_E) and clonal diversity (N_C:N_I) values. However, evidence for asexual reproduction does exist and this mode of reproduction may contribute to the clumped dispersion patterns and heterozygote deficiencies observed in established colonies. Dominance by sexual reproduction in this species is consistent with

population genetic studies conducted on other scleractinian coral species (Benzie et al. 1995, Yu et al. 1999, Ng and Morton 2003).

While my data provides evidence of asexual reproduction, whether fragmentation of branches with subsequent reestablishment immediately adjacent to the parent is enough to cause the clumped adult distribution is unknown. Other causes of aggregated population structure were not the focus of this study, but preferential settlement, post-settlement mortality, or kin association could certainly be contributors to this dispersion pattern in *O. arbuscula*. Preferential settlement of coral larvae can be induced by chemicals emitted by other species, such as coralline red algae (Morse et al. 1988). Marine larvae may also cue on specific benthic topography or local hydrodynamics that they assess by benthic-probing behavior (Havenhand and Svane 1991, Sebens and Johnson 1991, Mullineaux and Garland 1993, Altieri 2003). This scenario is plausible, because *O. varicosa* larvae exhibit benthic-probing behavior (Brooke and Young 2003) and *O. arbuscula* adults have increased feeding success in microhabitats with greater flow speeds over the bottom (Piniak 2002). Differential mortality may act on randomly settling *O. arbuscula* recruits, because suitable substrate within the Georgia reef habitat is very patchy. Association of kin may limit dispersal and increase fusion between related clonal individuals (Shields 1982), because survivorship (Sebens 1983, Hughes and Jackson 1985) and the onset of reproduction (Fadlallah 1983, Sebens 1983) is often size dependent. *Oculina arbuscula* populations do have limited dispersal and aggregated populations, but preliminary results of the settlement study show no evidence of fusion between recruits that settle and grow close together (Gleason, unpublished).

Genetic relatedness of adult colonies was also tested using a histocompatibility assay, but the results were inconclusive. Histocompatiblity studies are historically controversial. For example, histocompatibility assays showed that hydroids growing on the shells of hermit crabs represented kin aggregations (Hart and Grosberg 1999), but such a result could not be reconstructed in a later study using the same species (Nicotra and Buss 2005). Some scientists have even challenged whether self-recognition occurs at all because clonal genotypes are not a necessary prerequisite for fusion in many species (Stoddart et al. 1985). Therefore, inconsistencies between the results of the histocompatibility assay and those of the allozyme electrophoresis in *O. arbuscula* could be due to histocompatibility sensitivity to self as well as genetically different relatives. Other plausible explanations for incongruity in the histocompatiblity assay may be that the allorecognition locus in *O. arbuscula* is not able to discern between individuals or that the experiment did not run long enough to elicit reliable fusion or rejection responses.

Local recruitment of *O. arbuscula* is influenced by both biological and physical factors. Once again, using the reproductive biology of *O. varicosa* as a proxy for *O. arbuscula*, it can be surmised that *O. arbuscula* probably has a relatively short planktonic phase (between a week to 21+ days) compared to other marine species, such as fish (months) or lobsters (a year or more), perhaps resulting in more limited dispersal abilities (Brooke and Young 2003, Steneck 2006). It is also probably that, like other scleractinians (Mundy and Babcock 2000), *O. arbuscula* larvae use vertical swimming behavior to migrate between stratified ocean currents. They also probably exhibit benthic probing behavior which can be used for microhabitat discretion (Brooke and Young

2003). It has also been suggested that negatively buoyant eggs in corals, like the eggs of *O. varicosa*, help facilitate more local recruitment (Kojis and Quinn 1981a, Ayre and Hughes 2000). The timing of gamete release is unknown for *O. arbuscula* and may occur many times a year as indicated by year-round settlement on tiles (Gleason, unpublished), however *O. varicosa* spawns annually in late August or early September (Brooke and Young 2003). Spawn time is important for physical factors influencing larval dispersal, including speed and direction of currents, which can change throughout the year.

Ocean currents are a major determinant of marine larval dispersal. Currents off the Georgia coast have not been well characterized, but a drift bottle study found primarily southerly flowing currents in fall and winter, northerly flowing currents in spring, and both south and north currents in summer (Bumpus 1955). To determine if local recruitment is a physical possibility for *O. arbuscula* larvae given offshore current speeds and directions, I used data from a current meter located on the Gray's Reef data buoy to graph the path of a passive particle traveling on Georgia currents. The simulated *O. arbuscula* larva traveled on Georgia currents for its 21 day planktonic period starting on September 9, 2005 (Figure 9). The larva was graphed as traveling along the bottom for the first nine hours after spawning, but subsequently moved up into the water column and traveled along the top three meters for the rest of its travel.

The larva traveled approximately 369.2 km in 21 days, but ended up only about 59.0 km south of its starting point (Figure 9). This approximation is based on the larval competency period of the tropical *O. varicosa*, but *O. arbuscula* larvae may travel farther because colder water temperatures delay larval settlement in some coral species (Edmunds et al. 2001). Though *O. varicosa* larvae were observed to settle after 21 days,

they exhibited benthic-probing behavior after 1-2 weeks (Brooke and Young 2003). At a traveling time of seven days, the larvae could move as far as 60.6 km or as little as 8.1 km from its starting position. Furthermore, with a shorter planktonic period and the tidal current moving primarily east to west, it is possible for larvae to travel more along latitudinal lines. More limited dispersal by latitude coincides with the genetic data of Nei's dendrogram that grouped the northern and southern populations separately, due partly to the northern populations lacking two alleles present in the southern populations.

It should be pointed out that this model of larval dispersal is only meant as an approximation. The path and distance of travel was generated from data received at only one point and certainly currents may differ at other points along the path. Though the larva was treated as a passive particle, marine larvae are no longer considered passive in their dispersal (Ogden 1997, Cowen et al. 2000); however, invertebrate larvae may be influenced more by ocean currents than fish larvae (Bellwood et al. 1998). Models that are more complete than this have found similar local retention results. A model including both biological and physical factors found local recruitment of individuals (Cowen et al. 2006) and a simulation model of neutrally buoyant larvae with planktonic dispersal between 10-20 days found the highest number of larvae retained (Black 1993). Therefore, I conclude that the prevailing currents off the Georgia coast could allow local recruitment of planktonic *O. arbuscula* larvae, and that this result is consistent with biological data indicating local population genetic structure and a clumped dispersion pattern in adult colonies.

TABLES AND FIGURES

Table 1. Georgia reef physical characteristics. Physical characteristics for the five Georgia reefs used in this study of *O. arbuscula* recruitment.

Population	Reef Type	Substrate Type	Vertical Relief
JR	Ledge	Relic Scallop Shells	≤ 1 m
L6	Ledge	Aragonite	≤ 1.5 m
MS	Ledge	Aragonite	≤ 1 m
P1	Large Patch Rock	Aragonite	≤ 2 m
R2	Patch Rocks	Aragonite	≤ 0.5 m

Table 2. Histocompatibility assays. *Oculina arbuscula* branches put into contact either exhibited fusion or rejection response. The table shows the three populations (treatments and controls) used each summer for 2004 and 2005, the total number of surviving replicates, and the number of compatible vs. not compatible pairings occurred. A χ^2 test was used to determine if there was significantly more compatibility in the controls than in the treatments for each year.

2004 Populations	**n**	**Compatible**	**Not Compatible**
MS - MS	5	2	3
JR - JR	9	2	7
L6 – L6	6	3	3
MS - JR	8	3	5
MS – L6	11	0	11
JR – L6	2	0	2
Total Controls	20	7	13
Total Treatments	21	3	18
$\chi^2 = 2.38$, df = 2, p = 0.30			

2005 Populations	**n**	**Compatible**	**Not Compatible**
MS - MS	9	3	6
P1 - P1	15	2	13
R2 - R2	15	1	14
MS - P1	14	4	10
MS - R2	11	4	7
P1 - R2	11	3	8
Total Controls	39	6	33
Total Treatments	36	11	25
$\chi^2 = 2.46$, df = 2, p = 0.29			

Table 3. Allele frequencies for *O. arbuscula*. Allele frequencies at six polymorphic enzyme encoding loci for populations of *O. arbuscula* collected from 5 reefs in Georgia.

Locus	JR	L6	MS	P1	R2
GPI					
115	0.114	----	0.143	----	0.014
108	0.143	0.207	0.100	0.015	0.029
100	0.457	0.276	0.629	0.485	0.543
94	0.286	0.345	0.114	0.409	0.329
87	----	0.172	0.014	0.091	0.086
PGM					
110	0.157	0.121	0.100	0.061	0.186
105	----	----	0.043	0.030	0.171
100	0.457	0.655	0.471	0.364	0.357
94	0.300	0.190	0.214	0.455	0.100
87	0.086	0.035	0.171	0.091	0.186
MPI					
108	0.171	0.310	0.057	0.030	0.171
100	0.757	0.586	0.871	0.742	0.657
88	0.071	0.103	0.071	0.227	0.171
MDH					
113	0.329	0.155	0.371	0.409	0.400
105	0.029	0.138	0.228	0.197	0.200
100	0.614	0.500	0.371	0.288	0.371
91	0.029	0.207	0.029	0.106	0.029
CK					
138	----	----	0.200	0.015	0.029
111	0.400	0.310	0.329	0.258	0.129
100	0.329	0.517	0.324	0.409	0.557
84	0.271	0.172	0.157	0.318	0.286
AD					
114	0.243	0.431	0.451	0.424	0.486
100	0.671	0.397	0.357	0.515	0.514
85	0.086	0.172	0.185	0.061	----
(n)	(35)	(29)	(35)	(33)	(35)

Table 4. Nei's genetic distance, F_{ST}, and number of effective migrants. Nei's unbiased genetic distance, D, for five *O. arbuscula* populations off the coast of Georgia are above the diagonal. Lower values indicate greater genetic similarity. Measures of genetic difference between Georgia populations, F_{ST}, are below the diagonal with the mean number of effective migrants (N_m in parentheses) between pairs of populations. * $p < 0.05$

Population	**JR**	**L6**	**MS**	**P1**	**R2**
JR	----	0.082	0.076	0.066	0.086
L6	0.033* (7.35)	----	0.127	0.112	0.100
MS	0.031* (7.81)	0.056* (4.21)	----	0.072	0.076
P1	0.032* (7.56)	0.050* (4.75)	0.024* (10.17)	----	0.047
R2	0.034* (7.10)	0.041* (5.85)	0.032* (7.56)	0.012 (20.58)	----

Table 5. Heterozygosity and Hardy-Weinberg. The total number of alleles in Georgia *O. arbuscula* populations from the six polymorphic loci, observed and expected heterozygosity for each of the populations (two monomorphic loci included in calculation), and a test for goodness of fit, using the William's correction for small sample size. All populations are out of Hardy-Weinberg equilibrium (Chi square test for all populations, $\chi^2 \geq 26.89$, df = 1-15, $p < 0.03$). The coefficient D_S indicates populations out of Hardy-Weinberg equilibrium due to an observed surplus (positive value) or deficit (negative value) of heterozygotes.

Pop.	Mean number of alleles/ locus	Heterozygosity		G-test (df = 1)	p-value	D_S
		Obs.	Exp.			
JR	3.5	0.11	0.43	47.81	< 0.001	- 0.74
L6	3.5	0.13	0.47	24.46	< 0.001	- 0.72
MS	4	0.13	0.45	49.84	< 0.001	- 0.71
P1	3.8	0.10	0.45	42.95	< 0.001	- 0.78
R2	3.8	0.11	0.46	36.36	< 0.001	- 0.76

Table 6. F-statistics for *O. arbuscula* populations. F-statistics by locus for five Georgia populations of *O. arbuscula* (F_{ST}: standardized genetic variance between populations; F_{IS}: standardized genetic variance within populations). * $p < 0.05$

Locus	F_{ST}	F_{IS}
GPI	0.037*	0.860*
PGM	0.035*	0.815*
MPI	0.036*	0.873*
MDH	0.032*	0.715*
CK	0.034*	0.613*
AD	0.030*	0.577*
Mean	0.034*	0.737*

Table 7. Clonal diversity and genotypic diversity. Measures of clonal diversity and genotypic diversity to determine the amount of asexual reproduction occurring within Georgia *O. arbuscula* populations. $\mathbf{N_I}$: number of individuals, $\mathbf{N_C}$: number of unique multilocus genotypes (clones), $\mathbf{G_O}$: observed genotypic diversity, $\mathbf{G_E}$: expected genotypic diversity

Population	$\mathbf{N_I}$	$\mathbf{N_C}$	$\mathbf{N_C:N_I}$	$\mathbf{G_O}$	$\mathbf{G_E}$	$\mathbf{G_O:G_E}$
JR	35	23	0.66	16.3	19.0	0.86
L6	29	21	0.72	17.2	19.3	0.89
MS	35	23	0.66	17.3	18.2	0.95
P1	33	26	0.79	23.2	35.1	0.66
R2	35	26	0.74	19.4	18.4	1.05

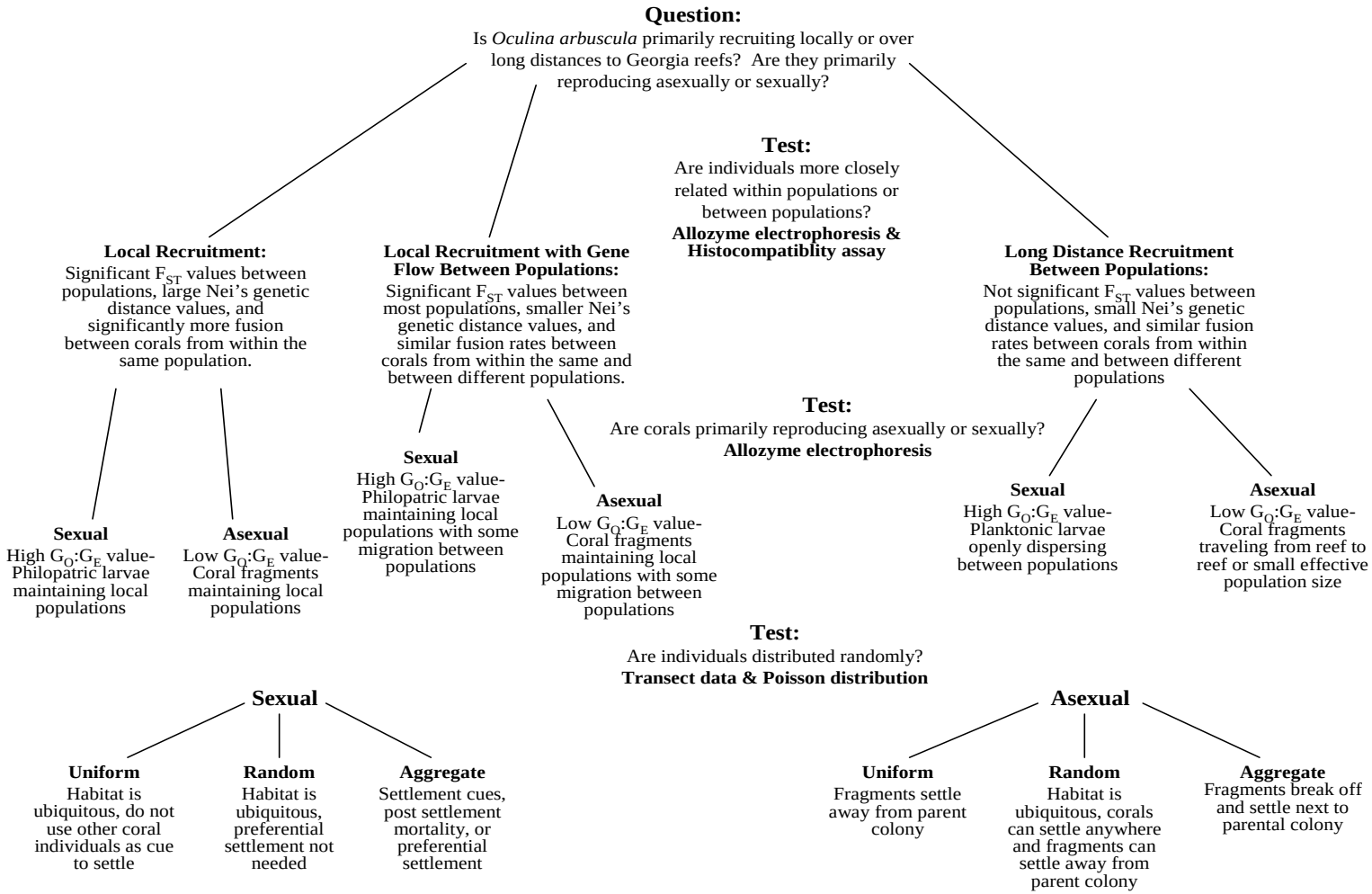

Figure 1. Thesis flow chart. Flow chart of questions and the possible outcomes from the various experiments and surveys involved in this study. Different combinations of results will lead to different outcomes.

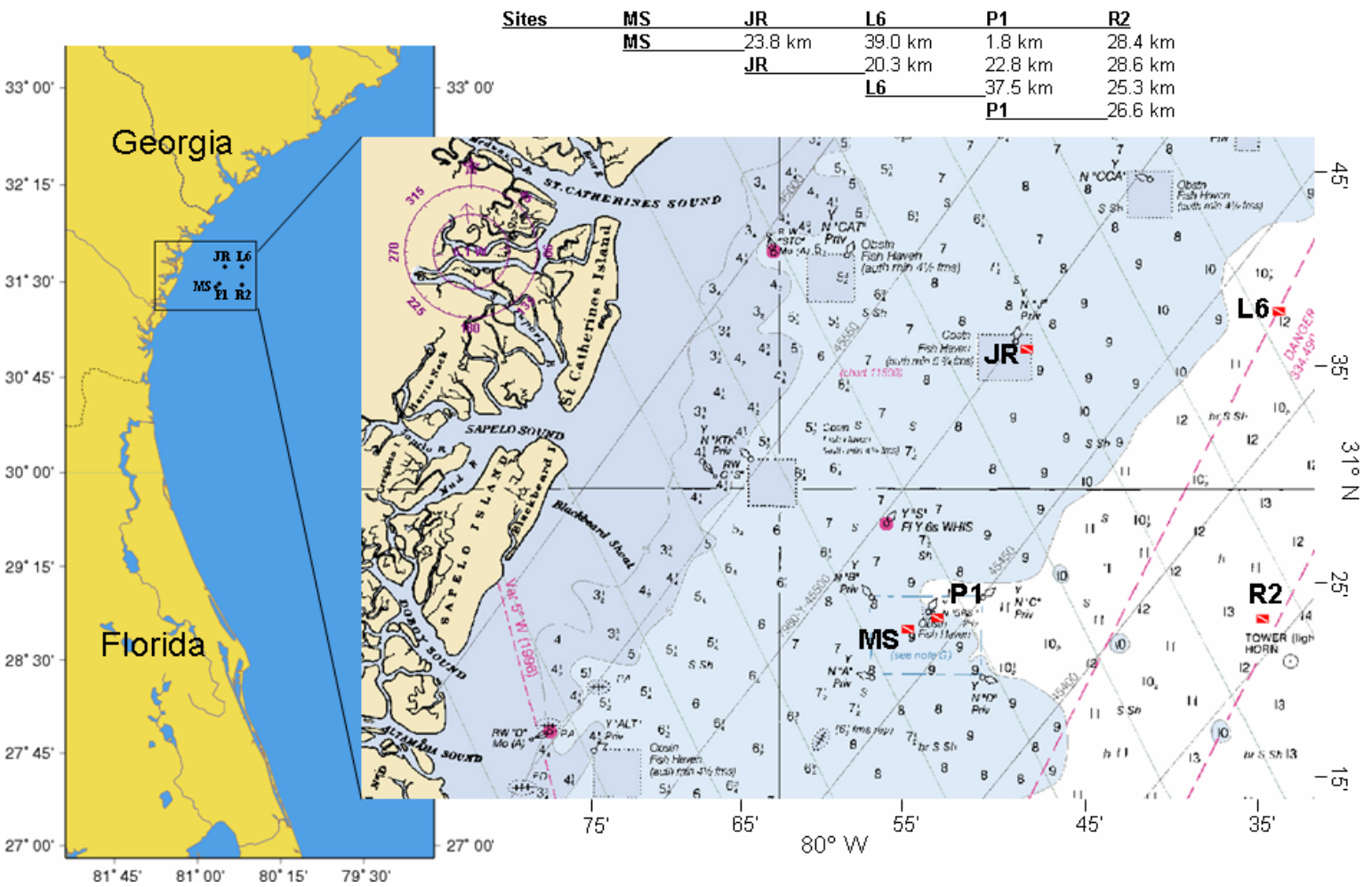

Figure 2. Chart of sampling sites in coastal Georgia. *Oculina arbuscula* sample sites in relation to the coast of Georgia. Abbreviations for sites as follows: **MS**- Gray's Reef Monitoring Site, **P1**- Gray's Reef Patch #1, **JR**- J-Reef, **L6**- Ledge #6, **R2**- R2 Tower hard bottom. Above the chart is a table of distances between collection sites.

Histocompatibility acceptance

Histocompatibility rejection

Figure 3. Histocompatiblity assays. Acceptance and rejection responses between paired corals. In the acceptance response, the coral skeleton and tissue are fused at the circled point of contact. In the rejection response, there is a dark band of tissue necrosis at the circled point of contact.

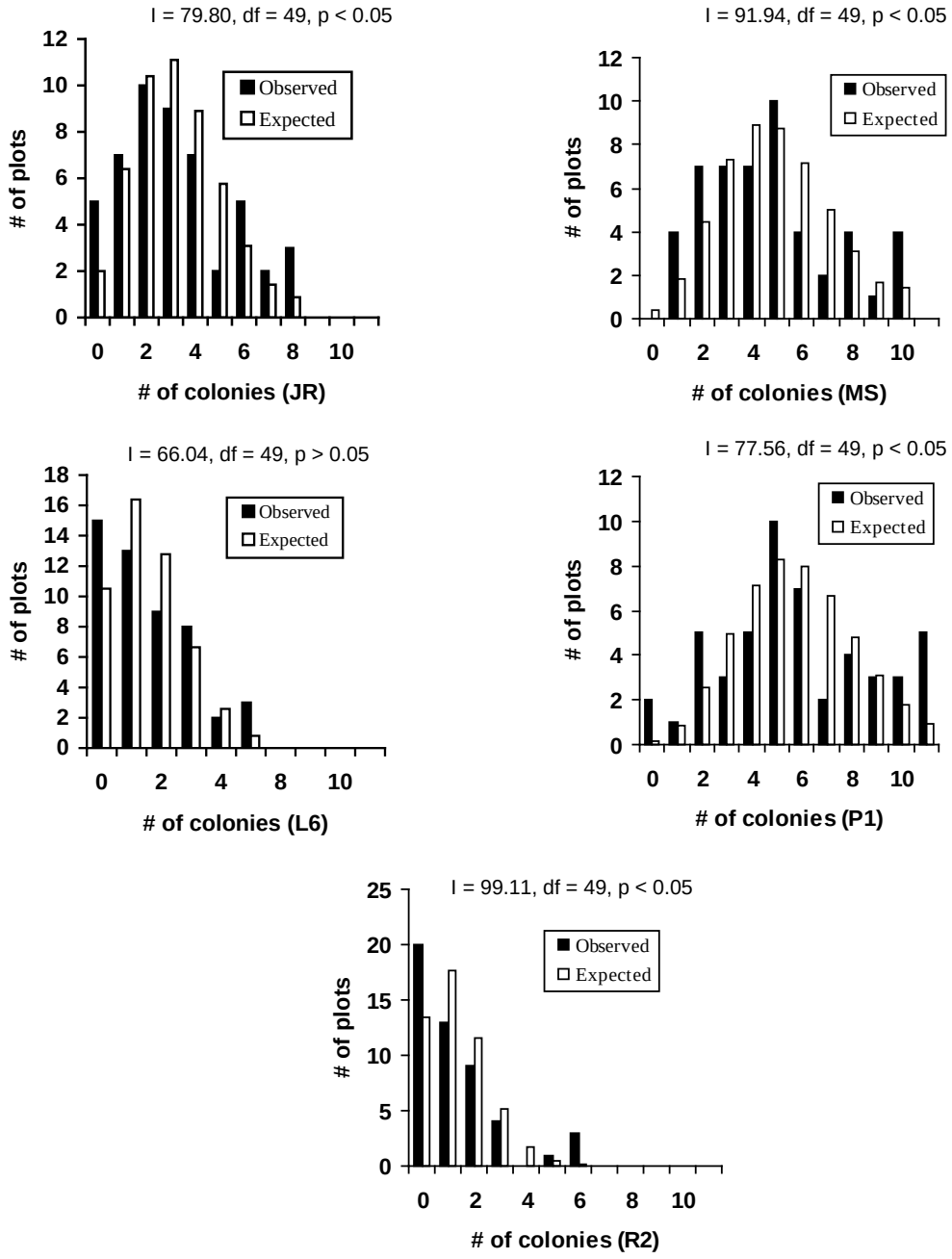

Figure 4. Frequency distributions of adult *O. arbuscula*. Frequency distributions of *O. arbuscula* colonies found in 1 m² plots at the five reefs studied off the Georgia coast. Expected frequencies of adult corals per plot (white bars) were calculated using a Poisson distribution.

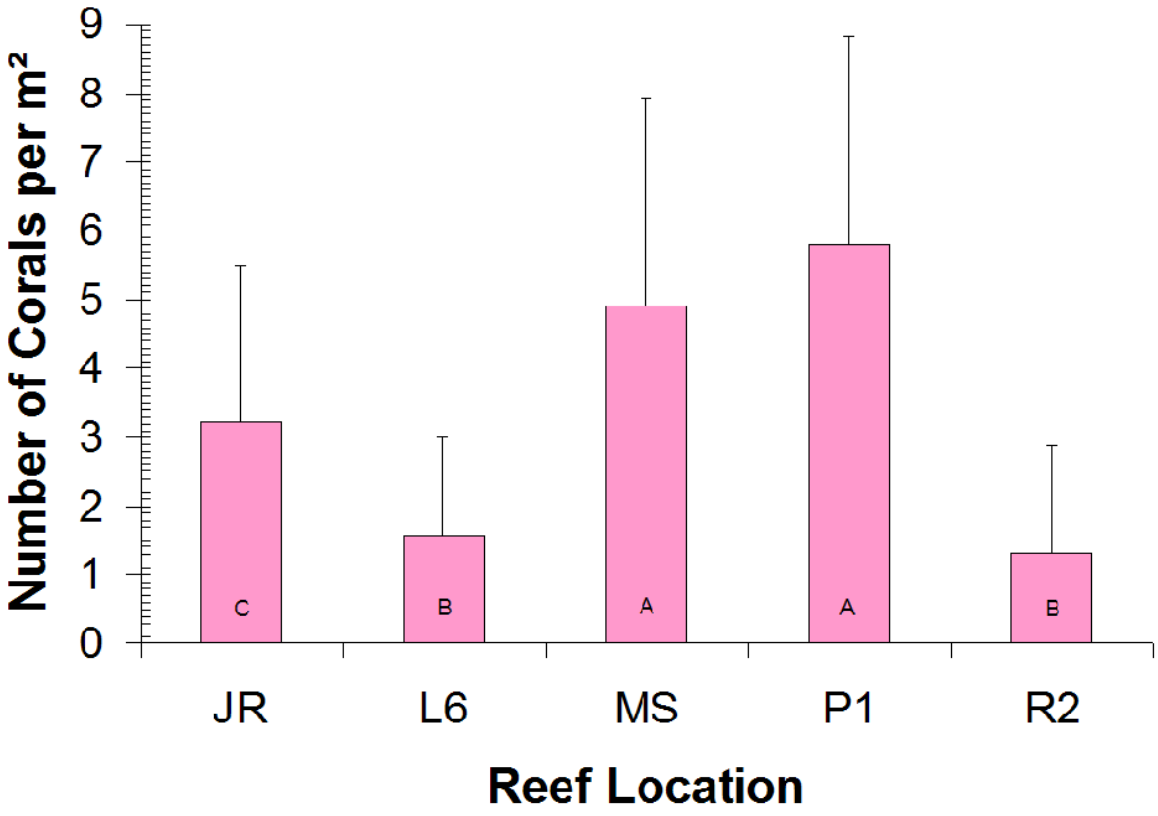

Figure 5. Colonies of *O. arbuscula* per 1 m² plots. Mean number of *O. arbuscula* colonies per 1 x 1m plot at five Georgia reefs. Error bars represent standard error (n = 50 plots at each site). Bars with the same interior letters are not significantly different (Tukey-Kramer, $\alpha = 0.05$).

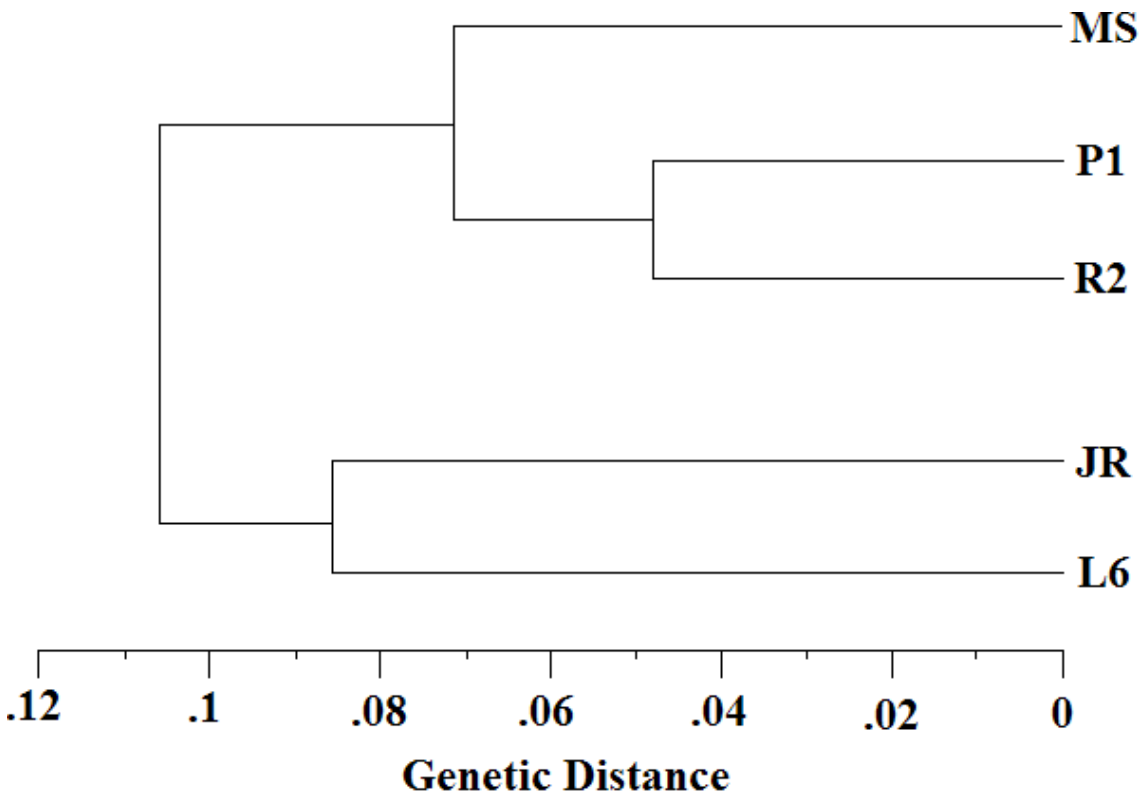

Figure 6. Nei's genetic distance UPGMA dendrogram. UPGMA (unweighted pair-group method using arithmetic averages) dendrogram showing genetic relationships of *O. arbuscula* populations on Georgia reefs based on values for Nei's unbiased genetic distance.

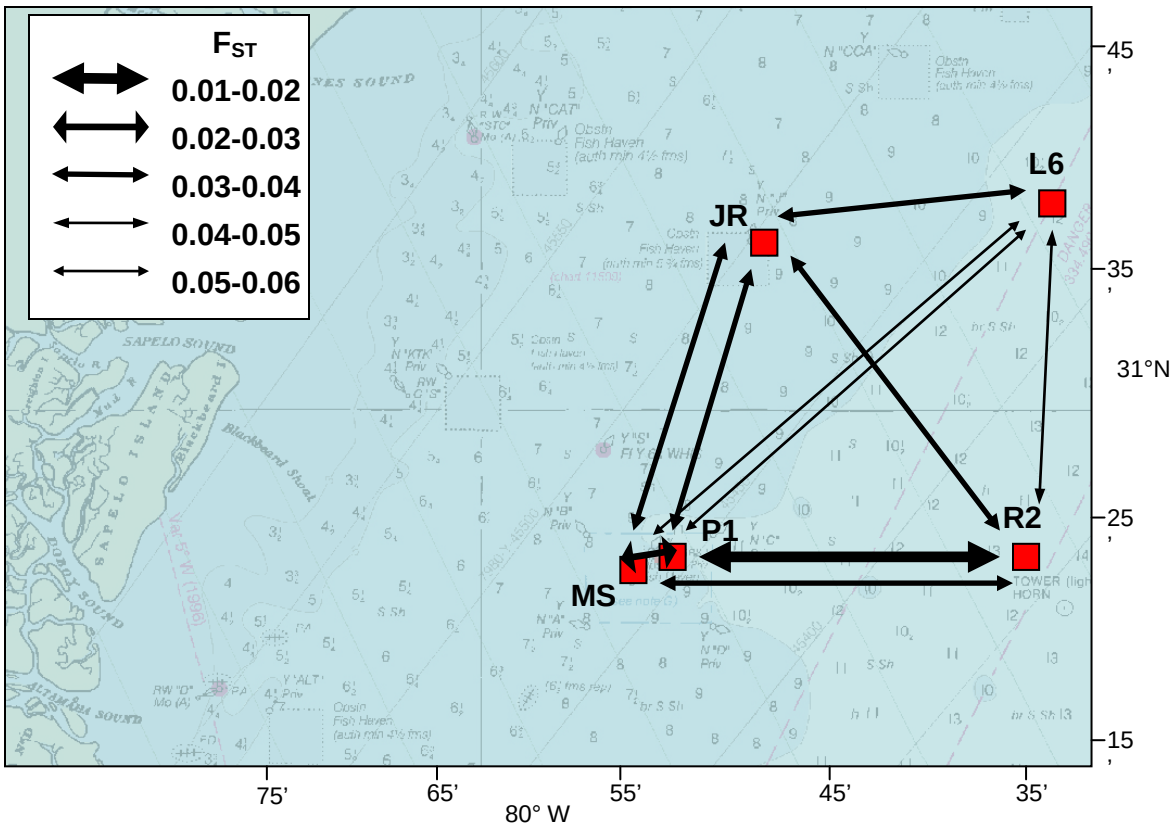

Figure 7. Genetic differences versus geographic distances. Genetic differences (F_{ST}) relative to locations for *O. arbuscula* populations off the Georgia coast. The thickness of the arrows connecting the populations indicates the genetic distance between them. Thicker arrows denote more gene flow between populations.

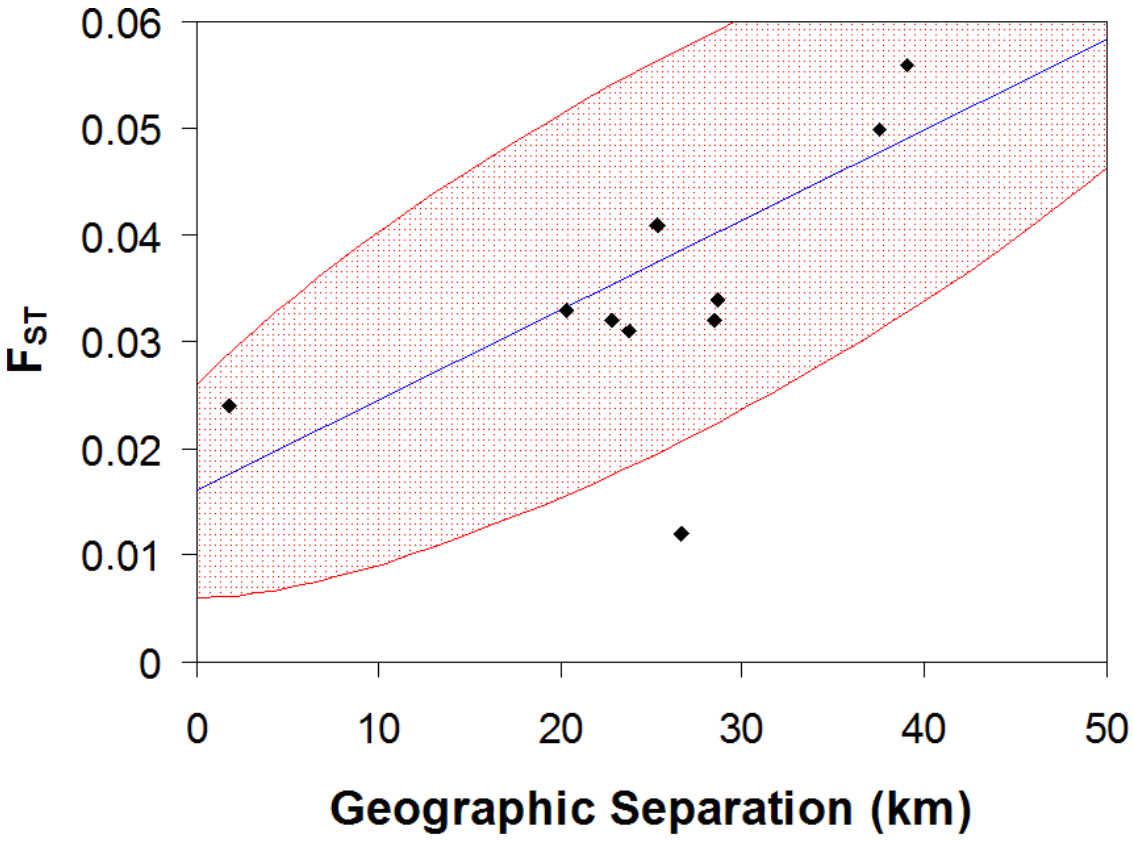

Figure 8. Regression of genetic differences versus geographic distances. Pairwise F_{ST} values between sample sites showing genetic differentiation of Georgia *O. arbuscula* populations as a function of distance between reefs. A bivariate normal ellipse (p = 0.99) was used to determine if there was an outlier. The outlier was removed and the regression line resulting from the remaining nine points was highly significant ($R^2 = 0.70$, df = 7, p = 0.005). Even when this outlier is included in the regression there is a trend for genetic similarity to be inversely related to distance ($R^2 = 0.39$, df = 8, p = 0.056).

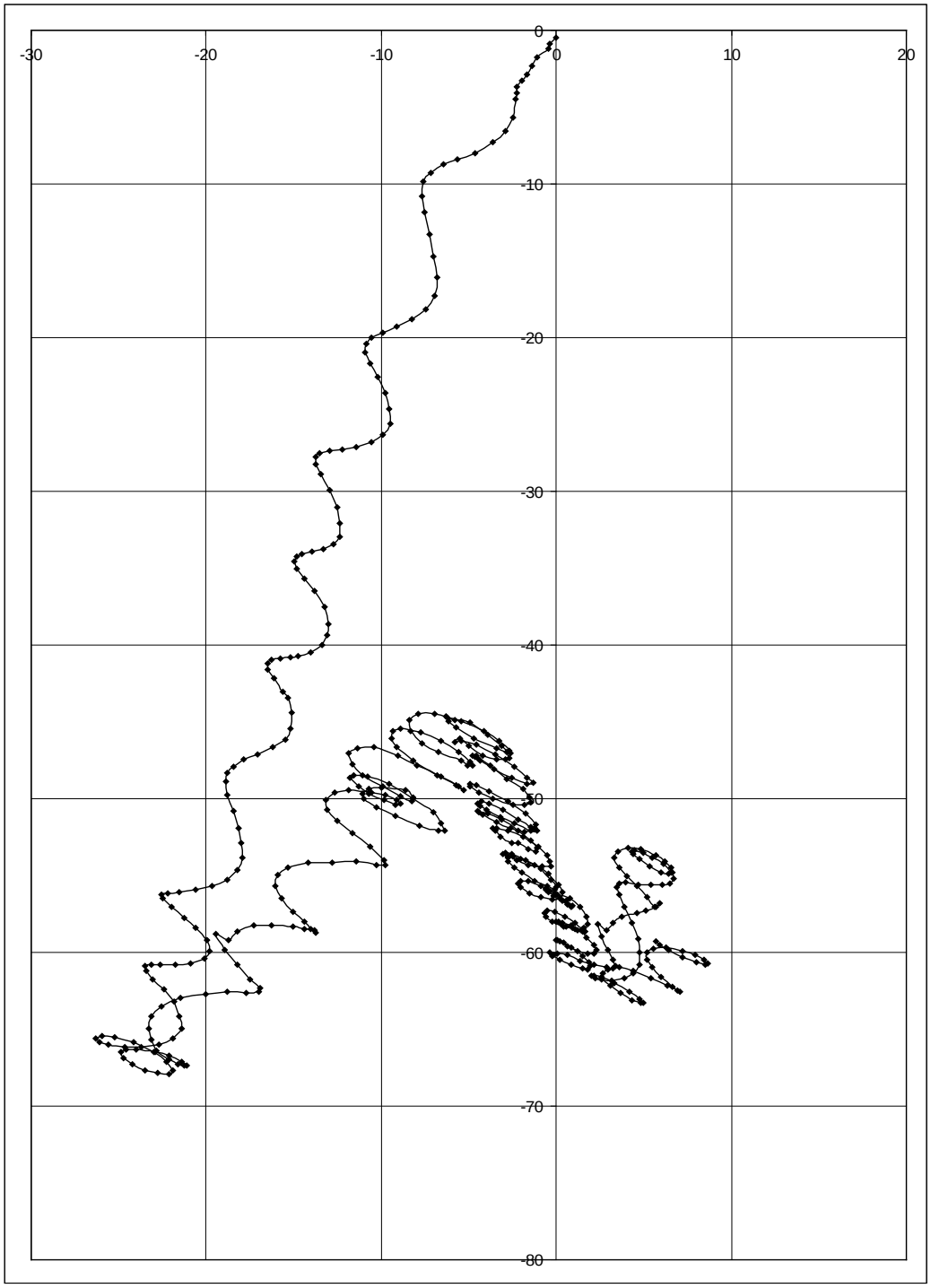

Figure 9. Passive larval travel in Georgia currents. Dispersal of a passive particle in oceanic currents observed at Gray's Reef National Marine Sanctuary traveling for twenty one days from September 9, 2005 to September 30, 2005. Travel of the particle was modeled using averages of current speed and direction taken from the Gray's Reef data buoy. Each point on the path is distance traveled in one hour. The grid is marked every 10 km.

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APPENDICES

Appendix A. Allozymes screened for electrophoresis. Allozymes screened in *O. arbuscula*, their enzyme commission numbers (EC), and whether or not they exhibited polymorphism.

Allozyme	Full name	EC Number	Banding pattern
AD	Aldehyde dehydrogenase	1.2.1.3	Polymorphic
ADH	Alcohol dehydrogenase	1.1.1.1	None
AK	Adenylate kinase	2.7.4.3	None
AO	Aldehyde oxidase	1.2.3.1	None
CK	Creatine kinase	2.7.3.2	Polymorphic
FUM	Fumarate hydratase	4.2.1.2	Monomorphic
G6PDH	Glucose-6-phosphate dehydrogenase	1.1.1.49	None
GPI	Phosphoglucose isomerase	5.3.1.9	Polymorphic
HK	Hexokinase	2.7.1.1	Monomorphic
IDH	Isocitrate dehydrogenase	1.1.1.42	None
LDH	Lactate dehydrogenase	1.1.1.27	None
MDH	Malate dehydrogenase	1.1.1.37	Polymorphic
ME	Malate dehydrogenase NADP+	1.1.1.40	None
MPI	Mannose-6-phosphate isomerase	5.3.1.8	Polymorphic
6PGDH	6-Phosphogluconate dehydrogenase	1.1.1.44	None
PGM	Phosphoglucomutase	5.4.2.2	Polymorphic
SOD	Superoxide dismutase	1.15.1.1	None
XDH	Xanthine dehydrogenase	1.1.1.204	None

Appendix B. Linkage disequilibrium. Linkage disequilibrium values for all locus pairs in *O. arbuscula* populations off the Georgia coast. Probability values are based on 90,000 permutations.

	MS	JR	L6	P1	R2
loc-GP X loc-PG	0.67605	0.02006	0.54977	0.13973	0.41390
loc-GP X loc-MP	0.23640	0.05700	0.02981	0.06936	0.02938
loc-GP X loc-MD	0.07560	0.00657	0.03428	0.35292	0.06736
loc-GP X loc-CK	0.00051	0.00218	0.21730	0.48951	0.07993
loc-GP X loc-AD	0.02808	0.07118	0.09697	0.01863	0.34085
loc-PG X loc-MP	0.53757	0.64943	0.95203	0.83160	0.00980
loc-PG X loc-MD	0.01160	0.00005*	0.09190	0.40886	0.00009
loc-PG X loc-CK	0.07793	0.01710	0.04263	0.00147	0.13236
loc-PG X loc-AD	0.57852	0.00072	0.00984	0.42971	0.00572
loc-MP X loc-MD	0.10334	0.25916	0.08817	0.88124	0.02108
loc-MP X loc-CK	0.44268	0.11997	0.20664	0.27484	0.14813
loc-MP X loc-AD	0.16872	0.01317	0.05104	0.16370	0.13690
loc-MD X loc-CK	0.00743	0.01004	0.23331	0.17437	0.21770
loc-MD X loc-AD	0.09039	0.03763	0.08538	0.18110	0.00438
loc-CK X loc-AD	0.00876	0.26638	0.01401	0.25198	0.16043