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Offshore oil and gas platforms as stepping stones for expansion of coral communities: a molecular genetic analysis

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OFFSHORE OIL AND GAS PLATFORMS AS STEPPING STONES FOR EXPANSION OF CORAL COMMUNITIES: A MOLECULAR GENETIC ANALYSIS

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ABSTRACT

The northern Gulf of Mexico (GOM) is one of the most productive oil and gas exploration areas in the world, currently containing approximately 3,800 offshore platforms. These platforms serve as artificial reefs in shallow water, which until their introduction was nearly devoid of shallow hard substrata. The question is raised whether this newly available substrate could help expand coral populations in the GOM. In this study, I examined adult scleractinian corals on oil and gas platforms in the northern GOM, in the vicinity of the Flower Garden Banks (FGB; approximately 180 km SE of Galveston, Texas) and attempted to determine the degree of genetic affinity among the natural and platform populations there. Adult coral tissue samples were collected from seven platforms surveyed in the region of the FGB at a depth range of 0-30 m. The three most abundant scleractinian, hermatypic species were sampled: *Madracis decactis*, *Diploria strigosa*, and *Montastraea cavernosa*. Genetic variation was revealed by Amplified Fragment Length Polymorphisms (AFLPs), a DNA-fingerprinting technique based on the polymerase chain reaction (PCR). This tool successfully distinguished between closely related colonies derived from populations on different platforms and on the two Flower Garden Banks. AMOVA analyses indicated that the East and West FGB were homogeneous for *Madracis decactis* and *Diploria strigosa*; however, the *Montastraea cavernosa* populations at the two banks were significantly different. Randomized data sets of two *Madracis decactis* populations were run with AFLPOP using a minimum log-likelihood difference of zero and one. These analyses determined that a log-likelihood difference of one is a more conservative and more reliable option, and all subsequent analyses were run using this setting. AFLPOP analyses showed that *Montastraea cavernosa* at the two banks was highly self-contained, indicating a possible high degree of self-seeding with regard to this species. It

appears that *Madracis decactis*, a brooding species, is highly effective at dispersing to neighboring habitats over distances of kms to tens of kms. By comparison, *Diploria strigosa* and *Montastraea cavernosa*, both broadcasting species, are not. They may be more effective at larger scale dispersal, but this remains to be demonstrated.

INTRODUCTION

Since the introduction of oil and gas platforms into the Gulf of Mexico (GOM), platforms have been serving as a substrate for colonization of a wide variety of marine organisms (corals, fish, and invertebrates). Relatively little is known about the distribution of hermatypic corals inhabiting platforms near the Flower Garden Banks (FGB; Bright et al., 1991; Gittings et al., 1992; Fenner, 2001). Even less is known about the connectedness of coral populations in this region (Snell et al., 1998; Brazeau et al., submitted). The research objective of this study is to determine the degree of genetic affinity among populations of the three most abundant scleractinian adult coral species found on a set of offshore platforms in the vicinity of the FGB and compare them with populations on the FGB.

In order to help the reader fully understand the role of platforms on the shelf, I will provide information on platforms as artificial reefs, the FGB, and reproductive strategies of the three coral species examined here. I will also review information on coral larval dispersal and make inferences about currents on dispersal in the northern GOM. In addition, I will provide information about the genetic techniques used in this study and compare them with a variety of other similar techniques used today, in particular comparing their effectiveness for revealing certain population genetic characteristics. Lastly, I will provide information on inferring larval dispersal from genetic data.

Offshore Platforms

The first offshore platform was installed in 1938, 2.4 km off the Louisiana coast (Gallaway 1981, Gallaway and Lewbell, 1982). After World War II, in 1947, a significant amount of offshore exploration had occurred, beginning with Ship Shoal Block 32 field being discovered 19 km off the coast of Louisiana (Winfield, 1973; Gallaway and Lewbell, 1982). The

field began to rapidly expand in 1953, with peak installation of production platforms occurring in 1956. Today there are approximately 3,800 platforms in the northern GOM (Wilson and Van Sickle, 1987; Knott, 1995; Dauterive, 2000).

In a region typically characterized by terrigenous, sandy muds, resulting in little habitat diversity (Shideler, 1978; Rezak et al., 1985; Scarborough-Bull, 1989; Brown et al., 1999), these platforms extend through the euphotic zone, providing hard substrate in open water (Shinn, 1973, 1974), that would otherwise be unavailable to sessile, epibenthic organisms, allowing them to expand their normal range of distribution. The first studies of the biological communities associated with platforms began in 1955 by Gunter and Geyer, who described the biofouling community growing on the legs of an oil platform in the northern GOM (see also Gallaway and Lewbel, 1982). In 1974, Shinn was the first to describe the composition and vertical zonation of fish around Louisiana offshore platforms. Compared to the soft-bottom areas nearby, nearly 20 to 25 times more fish were found under and near oil platforms (Shinn, 1973, 1974; Driessen, 1989). These studies helped to show the ecological impact of platforms in the Gulf.

In addition, platforms provide important habitat for invertebrates (Shinn, 1973; Driessen, 1989, Scarborough- Bull, 1989). A 200 ft. platform jacket can provide several acres of hard substrate, which encourages the growth and settlement of sessile algae, barnacles, mussels, anemones, and other attached organisms (Shinn, 1974; Driessen, 1989).

Several coral species (*Diploria* spp., *Porites astreoides*, *Madracis decactis*, *M. asperula*, and *Millepora alcicornis*) have also been found on various platforms in the GOM (Bright et al., 1991; Sammarco and Atchison, 2004; Sammarco et al., 2003, 2004). Thus, the possibility was introduced that these oil and gas platforms might be environmentally, as well as economically important. Nearly 70% of all sport fishing trips greater than three miles from the coast are to oil

and gas platforms, accounting for a significant portion of the $640 million from licensed salt-water fishing (Driessen, 1989). Platforms in the Gulf produce 25% of the natural gas in the United States, 13% of its oil, and represent the largest artificial reef system in the world (Kasprzak, 1998; Stanley and Wilson, 2003). In addition, the GOM is also the source of 98% of the gas and 91% of the oil derived from the nation's Outer Continental Shelf (Dauterive, 2000).

Although there are currently thousands of platforms operating in the GOM (Reggio, 1987; Scarborough-Bull and Kendall, 1994; Knott, 1995), a decline in this number is expected over the next few decades. From 1973 to 1993, a total of 1,115 platforms were removed from the GOM (Kasprzak, 1998). It is expected that 29% of offshore structures will be removed over the next quarter century, resulting in the loss of a great deal of hard substrate (Poruban, 2001). As a result, this reduction in artificial reef habitats may have long-term negative consequences on fish populations as well as numerous encrusting organisms that depend on the hard substrate for survival (Tubb et al., 1980; Grossman et al., 1997; Lindberg, 1997; Kasprzak, 1998; Wilson et al., 2001).

Impacts can be diminished if some of the platforms are converted into artificial reefs. A joint federal/state "Rigs-to-Reef" Program allows for approximately 10% of the platforms scheduled for decommissioning to be converted in to artificial reefs (Poruban, 2001). Since platforms have a potential expected life-span of 300 years (Quigel and Thornton, 1989), ecological communities associated with them would be permitted to develop fully, into climax communities, if left undisturbed (Clements, 1916; Odum, 1969; 1971; Saito et al., 1976; Taniguchi, 1996).

Artificial Reefs

The first documented cases of the United States building artificial reefs were in the 1830's. Wooden shelters constructed of logs were floated out to the desired location and sunk by heavy rocks to replace trees that had fallen into South Carolina coastal waters naturally that had provided productive fishing areas at one time (Christian et al., 1998). The first scientific studies of artificial reefs began in 1958 off the coast of California. In this study, artificial reefs were constructed out of old streetcars and automobiles. It was later found that a more durable material such as automobile tires was needed, which were easy to handle, low cost, and abundant (Shinn, 1973).

Today, many states have artificial reef programs in place. To further aid in the expansion of the artificial reef program, the National Fishing Enhancement Act (Title II of P.L. 98-623) was passed in 1984, which was designed to ensure responsible and effective construction of artificial reefs in United States waters. This Act imposed national standards, provides for a National Artificial Reef Plan (USDC, NMFS, 1985), and addresses the liability of a reef permittee as well as the types of material that can be used for reef construction (Quigel and Thorton, 1989).

These same effects have been created through the introduction of platforms into the GOM, which have had numerous effects on the marine communities there. Platforms provide the majority of all hard-bottom habitat off the Louisiana coast in waters less than 120 m deep (Driessen, 1989). A single platform in a 100 ft. of water can provide approximately two acres of hard substrate to the water column (Driessen, 1989; Shinn, 1974; Sammarco et al., 2004). These platforms have led to fish population explosions (Winfield, 1973; Daniel and Seward, 1975) and provided habitat for schools of reef fish in addition to the natural coral reefs and other

surrounding hard banks. As a result, a unique mixture of tropical reef and offshore pelagic fish species occur on platforms, supporting diverse fish life for both pelagic fish as well as demersal site-attached fish (Rooker et al., 1997). During the last 13 years, 151 platforms (Louisiana-94 and Texas-50) have been donated by oil and gas companies, serving as artificial reefs in the Gulf (Dauterive, 2000). Under current legislation, however, almost all of the other 3,800 structures will eventually be removed, creating damaging effects on the marine populations in this region (Shinn, 1974).

Flower Garden Banks

The Flower Garden Banks were declared a National Oceanic and Atmospheric Administration (NOAA) National Marine Sanctuary in 1992 (NOAA-NMS, 1992; Dokken et al., 1999, 2002). They are located in the NW GOM at the edge of the continental shelf, approximately 180 km southeast of Galveston, Texas (Bright et al., 1984, 1985). The FGB are located within the most active offshore oil and gas exploration and production area in the world (Dokken et al., 2002) and are comprised of two banks: the East Bank (27º54'32" N, 93º36' W) and West Bank (27º52'27" N, 93º48'47" W; McGrail et al., 1982; Rezak et al., 1983a, b; Bright et al., 1984, 1985). The banks are salt domes formed by the intrusion of salt plugs formed from Jurassic evaporate deposits, 15 km below the sea floor (Rezak et al., 1983a). Coral reefs have developed on their caps (McGrail et al., 1982; Bright et al., 1984; Dokken et al., 1999), which rise to within 18 m of the sea surface (Rezak et al., 1983a, b) and extend to depths of 100-150 m (Hagman et al., 1998a; Lugo-Fernandez et al., 2001). The East Flower Garden Bank (EFGB) has an area of 67 km^2, and the West Flower Garden Bank (WFGB) is twice as large with an area of 137 km^2. The distance between the two banks is 12 km (Rezak et al., 1983a, b, 1985; Dokken et al., 2002). The salinities at the surface of the reefs generally range between 34-36 with

temperatures varying from approximately 20ºC (mid-February) to a high of 30ºC (August), depending on the time of year (Rezak et al., 1983a; Bright et al., 1985; Dokken et al., 1999, 2002).

The FGB are unique because they are the northern-most coral reef system on the continental shelf of North America (Tresslar, 1974; Bright et al., 1984, 1985; Gittings et al., 1992; Dokken et al., 1999) and are separated by more than 600 km of open ocean from any other well-developed coral reefs (Hagman et al., 1998a; Sammarco et al., 2004). The FGB are productive (Rezak et al., 1985; Sammarco et al., 2003; Sammarco and Atchison, 2004), healthy reefs in comparison with other reefs in the Mexican GOM, Florida Keys, and Caribbean Sea. They are relatively isolated, with the closest reef system being Lobos-Tuxpan, located 13 km off Cabo Rojo, Mexico (Bright et al., 1985; Dokken et al., 2002), and approximately 640 km from the FGB (Fig. 1). The next closest reef system is the Campeche Bank reefs located 180 km off the Yucatan peninsula. Since the FGB is isolated from other coral reefs, the transmission of disease pathogens is relatively low (Dokken et al., 2002). Their distance from the mainland protects them from terrestrial impacts, and an 18 m water column serves as a buffer from extreme surface water temperatures (Dokken et al., 2002), which can induce bleaching (Fitt and Warner, 1995; Douglas, 2003; Lasker, 2003).

The upper 50 m are exposed to oceanic conditions favoring reef growth. These reefs are characterized by 20 species of hermatypic corals occurring at depths of 15-48 m depth (Bright et al., 1984, 1985; Dokken et al., 1999, 2002). These include: *Agaricia* spp., *Colpophyllia natans*, *Diploria strigosa*, *Madracis decactis*, *Montastraea annularis*, *M. cavernosa*, *Stephanocoenia* spp., and *Porites astreoides* (Gittings et al., 1992; Lugo-Fernandez et al., 2001).

The FGB, Bermuda, and Florida Middle Ground represent three separate, northern biogeogaphic locations for Atlantic coral reefs; diversity at each of these locations is low in comparison to Caribbean reefs, with the number of coral species being 20, 24, and 16, respectively (Rezak et al., 1983b; Bright et al., 1984). By comparison, the Caribbean has 64 sp.(Goreau and Wells, 1967; Wells, 1973), south Florida 51 sp., the Bahamas approximately 30 sp., and southern GOM 34 sp. (Wells, 1988).

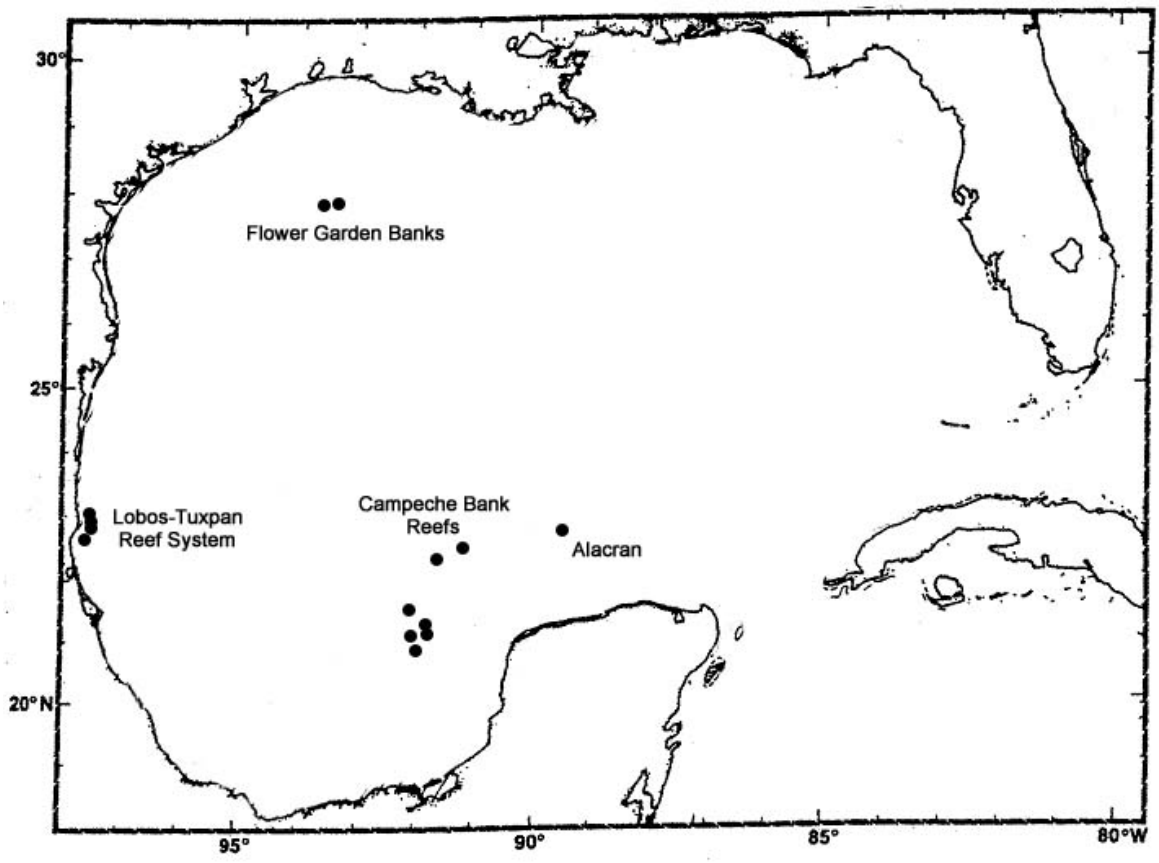


Figure 1. Map of the Gulf of Mexico depicting the nearest neighboring reefs to the Flower Garden Banks. The Lobos-Tuxpan reef system is located 13 km off Cabo Rojo, Mexico, and approximately 640 km from the FGB. The other nearest reef system, Campeche Bank reefs, is located about 180 km off the Yucatan Peninsula.

Other banks may also be found on the shelf in the northwestern GOM. Mid-shelf banks are defined as those banks that rise from depths of 80 m or less and have a relief of about 4 to 50 m (Rezak et al., 1985). These include Stetson, Claypile, Coffee Lump, Sonnier, Fishnet, and 32 Fathom. All of these banks have similar geological composition; they are outcrops of bedded Tertiary limestones, sandstones, claystones, and siltstones. Unlike the FGB, however, they are not true coral reefs. Although hermatypic corals may be found on Bright, Sonnier, Stetson, MacNeil Banks, Claypile, and Coffee Lump, coverage is sparse (Rezak et al., 1985; Lugo-Fernandez et al., 2001; Schmahl, 2003; Sammarco et al. 2004; Boland pers. obs.), and the substratum of these banks is not composed of calcium carbonate, accreted by corals as in the case of the FGB.

Coral Reproduction

Corals use a variety of modes of reproduction (Sammarco, 1982b; Harrison et al., 1984; Babcock et al., 1986; Harrison and Wallace, 1990; Kramarsky-Winter et al., 1997). The primary modes of sexual reproduction, however, are the brooding of planula larvae within the polyp or the broadcasting of gametes into the water column. Corals that use internal fertilization and develop larvae within their polyps are known as "brooders". With brooding, embryos develop within the parent for several weeks before they are released (Carlon, 1999). At this advanced stage of development, the larvae are competent to settle in ≥ 4 hrs (Fadlallah, 1983; Harrison et al., 1984; Harrison and Wallace, 1990; Carlon, 1999). Brooders typically become reproductive at ages much earlier (1-2 yrs.) than broadcast spawners (≥ 4 yrs.; Harrison et al., 1984; Harrison and Wallace, 1990). Brooders usually planulate monthly, releasing larvae up to 10 times per year (McGuire, 1998).

Most corals spawn eggs and sperm for external fertilization and development, which is called "broadcast spawning" (Harrison et al., 1984; Hagman et al., 1998a, b; Harrison and Wallace, 1990). One hundred fifty-seven scleractinian species worldwide are known to spawn gametes for external fertilization (Harrison and Wallace, 1990). With broadcast spawning, eggs and sperm are packaged together within gamete bundles and expelled through the polyp mouth in synchronous, annual spawning events. Gamete bundles are buoyant and float to the surface, where they break apart and fertilization occurs. Embryos that result from spawning require approximately 4 to 7 days to completely develop in the water column before they are competent to settle (Harrison and Wallace, 1990; Carlon, 1999). Broadcast spawning is usually seasonal, with spawning occurring during brief periods during the summer. Species utilizing this mode of reproduction are synchronized with lunar phases, and spawning generally occurs on the same lunar phase each year (Harrison and Wallace, 1990).

It was believed that mass spawning events occurred only in the Pacific as observed on the Great Barrier Reef in Australia. Since 1990, however, reports have documented such events throughout the Caribbean (Szmant, 1991; de Graaf et al., 1999; Sanchez et al., 1999; Levitan et al., 2004) and the FGB (Gittings et al., 1992; Boland, 1998; Hagman et al., 1998a, b). Bright et al. (1991) first documented mass spawning at the FGB in August 1990. Of the 20 species present, seven participate in annual mass spawning events including: *Colpophyllia natans*, *Diploria strigosa*, *Montastraea annularis*, *M. cavernosa*, and *Stephanocoenia intercepta* (Hagman et al., 1998a, b). These seven species make up approximately 82% of the FGB coral cover (Lugo-Fernandez et al., 2001). Mass spawning occurs on 7 to 10 evenings, following the full moon between 1 and 4 hrs after sunset (Hagman et al., 1998a) during August and occasionally in September, depending on mean sea surface temperatures (SSTs). These

spawning events seem to be synchronous with other Caribbean reefs, which are also correlated with lunar phase and maximum water temperature (Lugo-Fernandez et al., 2001).

Of the 20 coral species occurring at the FGB, three species were examined in this study: *Diploria strigosa*, *Madracis decactis*, and *Montastraea cavernosa* (Coelenterata, Scleractinia). Information on their authorities and reproductive modes are listed in Table 1.

Table 1. Scientific names of the three coral species collected with their authorities and modes of reproduction.

Scientific name	Authority	Family	Common name	Reproduction
Diploria strigosa	Dana, 1846	Faviidae	"Brain coral"	Broadcast spawner, participates in mass spawning (Soong, 1993; Gittings et al., 1992; Gittings et al., 1994; Hagman et al., 1998a, b; Bassim and Sammarco, 2003)
Madracis decactis	Lyman, 1859	Pocilloporidae	"Ten-rayed star coral"	Brooder (Diekmann et al., 2001; Vermeij et al., 2003)
Montastraea cavernosa	Linnaeus, 1767	Faviidae	"Large star coral"	Broadcast spawner, participates in mass spawning (Szmant, 1991; Gittings et al., 1992; Soong, 1993; Gittings et al., 1994; Acosta and Zea, 1997; Hagman et al., 1998a, b; Carlon, 1999)

Until recently, little was known about reproduction in *Madracis decactis*. It is now known, however, that it is a brooder (Diekmann et al., 2001; Vermeij et al., 2003), with planulae size twice as large as other *Madracis* spp. (Vermeij et al., 2003). In all *Madracis* spp., mature gametes of both sexes are present at the same time. Planular release occurs from March to

December, with maximum release occurring from September to November (Vermeij et al., 2003). In general, most colonies release planulae over consecutive days. There is no correlation between larval release and lunar cycle, but release does appear to be correlated with seawater temperature (Vermeij et al., 2003).

Larval Dispersal

Dispersal strategies for reproductive propagules vary widely between species. In terrestrial environments, there is a high potential for long-distance dispersal in plant propagules to occur. An example is plants that use wind in order to disperse pollen and seeds (Colwell, 1949, 1951; see Sammarco and Andrews, 1988, 1989; Tackenbery et al., 2003; Soons et al., 2004). Wind transport has also been known to carry microbes such as fungi, bacteria, and viruses across the Atlantic Ocean in African soil dust. Annually, millions of tons of African dust along with fungal spores are dispersed long distances by the trade winds, which takes on average 5-7 d (Shinn et al., 2000; Griffin et al., 2001, 2002; Kellogg and Griffin, 2003; Garrison et al., 2003).

In the marine environment, the closest analog to wind dispersal is ocean currents (Sammarco, 1994; Sammarco and Andrews, 1989). Like wind dispersal, ocean currents provide the potential for marine larvae to be transported great distances; however, dispersal in seeds *vs*. larvae is very different. Some species tend to establish themselves soon after release while others postpone metamorphosis, affording the opportunity for larvae, especially those of sessile marine invertebrates, to be widely dispersed (Olson, 1985; Willis and Oliver, 1990; Ayre and Hughes, 2000; Mora et al., 2001). It is this mechanism that is most important for short and intermediate distance dispersal (Jokiel, 1984).

Not all marine invertebrate larvae, however, develop into a swimming phase. Most benthic peracarid crustaceans brood their young until they develop into a crawling stage. Some gastropods (Highsmith, 1985; Hoskin, 1997; Tan, 1997), teredinid bivalves (Turner, 1966; Calloway and Turner, 1983; Hoagland and Turner, 1980; Sammarco, 1994), and corals (e.g. *Balanophyllia elegans*; Gerrodette, 1981) also produce crawling juveniles. Although these species lack a swimming dispersal phase, they exhibit a very broad geographic range (Highsmith, 1985; Sammarco, 1994).

There are a variety of mechanisms that sessile marine invertebrates use to disperse over long distances. Two of these mechanisms are floating and rafting (Jokiel, 1984; Highsmith, 1985). A variety of invertebrates such as *Pontogeneia* sp. (amphipod), *Transennella tantilla* (bivalve), and *Leptochelia dubia* (tanaid) brood and release benthic juvenile larvae, which have the potential to use flotation as a dispersal mechanism (Highsmith, 1985; Willis and Oliver, 1990).

It is also believed that many animals are frequently dispersed by rafting on algae and other flotsam. This provides organisms such as corals with an extended drift time, allowing them to span a large geographical range colonizing new locations in turn, later releasing gametes or larvae to expand over an even wider area (Jokiel, 1984; Highsmith, 1985; Willis and Oliver, 1990). Coral colonies have been found growing on objects such as logs, volcanic pumice, coconuts, and ship's hulls. Rafting occurs but is infrequent compared to larval dispersal (Willis and Oliver, 1990). Nevertheless, it provides an alternative to larval dispersal and has the potential to be of great importance by allowing long-range colonization of larvae, bridging wide gaps in the open ocean (Jokiel, 1984).

Other mechanisms that larvae utilize to disperse great distances are anthropogenic in nature. One is the ballast water of ships (Torchin et al., 2002). A variety of marine organisms have been carried across the ocean, introducing non-native species to a region (Grantham and Barrett-O'Leary, 1999; Hutchings et al., 2002). Some of these species include ascidians (Lambert, 2002), mussels (Kumpf et al., 1999), fish (Kumpf et al., 1999; Semmens et al., 2004) and corals (Cairns, 1994; Fenner, 2001).

There are two mechanisms that planula larvae can use in order to lengthen planktonic existence: extended larval competency, and oceanic circulation patterns, both supporting long-distance dispersal (Richmond, 1987). Olson (1985) found short-distance dispersal and direction of the ascidian species, *Didemnum molle*, was correlated with the prevailing water currents. With broadcast spawning, it is believed that the potential for larval dispersal can increase, and as a result, provide many adaptive benefits (Miller and Mundy, 2003). Some of these benefits include reducing competition for resources between parent and offspring, colonization of new habitats, increased gene flow, and genetic diversity (Olson, 1985; Miller and Mundy, 2003). By tracking coral spawn slicks, larvae have been found to disperse great distances during a 4-6 d period. It is believed, however, that the larval dispersal of broadcast spawning corals might not be as great as was once thought (Miller and Mundy, 2003). Dispersal among brooding species, which is dependent upon the larval characteristics and prevailing currents during the planktonic phase, may actually be more variable than among broadcast spawners (Harii and Kayanne, 2003). Data from this and sister studies suggest that brooders are effective at short distance dispersal, while broadcast spawners are adapted for longer-distance dispersal on and between reefs. Once planulae have developed, the potential for long-range dispersal between brooders and broadcasters are most likely comparable (Sammarco and Andrews, 1989). Overall, the

dispersal distance of larvae is most likely determined by the competency period of a species, larval position in the water column, and currents that deliver the larvae (Harii and Kayanne, 2003).

Physical Oceanography of the Region

The GOM is a semi-enclosed sea that consists of a basin 1.5 million km^2 in area (Lewis, 1992). Residence time of GOM water is short, approximately 100 years (Welsh and Inoue, 2000). The circulation processes in this region are highly variable and is characterized by the Loop Current (LC; Johnson et al., 1992; Ohlmann et al., 2001), Loop Current Eddies (LCEs; Johnson et al., 1992), and strong coastal jets (Fig. 2; Lewis, 1992; Oey, 1995). Both the LC and LCEs are large, energetic features with horizontal scales of several hundred kilometers (Johnson et al., 1992). The LC is a part of the North Atlantic's western boundary current that enters the Gulf through the Yucatan Channel and extends generally northward before exiting through the Florida Straits (Huh and Schaudt, 1990; Johnson et al., 1992; Sturges and Leben, 2000; Ohlmann et al., 2001). LC eddies are shed from the LC (Fig. 2) via instability processes (Johnson et al., 1992).

Ocean circulation is a key method of coral larval dispersal and is also considered an important aspect in coral evolution and biogeography (Lugo-Fernandez et al., 2001). The main driving force of circulation in the GOM beyond the region of the LC is from the vertical convergence of wind stress curl (Sturges, 1993; Oey, 1995; Ohlmann et al., 2001; Walker, 2001). The most prominent shelf circulation is considered to be cyclonic with dominant flow to the west nearshore and eastward on the outer shelf and shelf break, except during July-August. Nearshore flow is a result of west/southward wind that prevails most of the year except in summer; however, eastward flow on the outer shelf is more uncertain. During the summer,

westward shelf transport is small due to south winds (Oey, 1995). A seasonal western boundary current (WBC) occurs between 23° and 26°N with strongest flow in July-August, following one to two months after the maximum wind stress curl (Sturges, 1993; Oey, 1995).

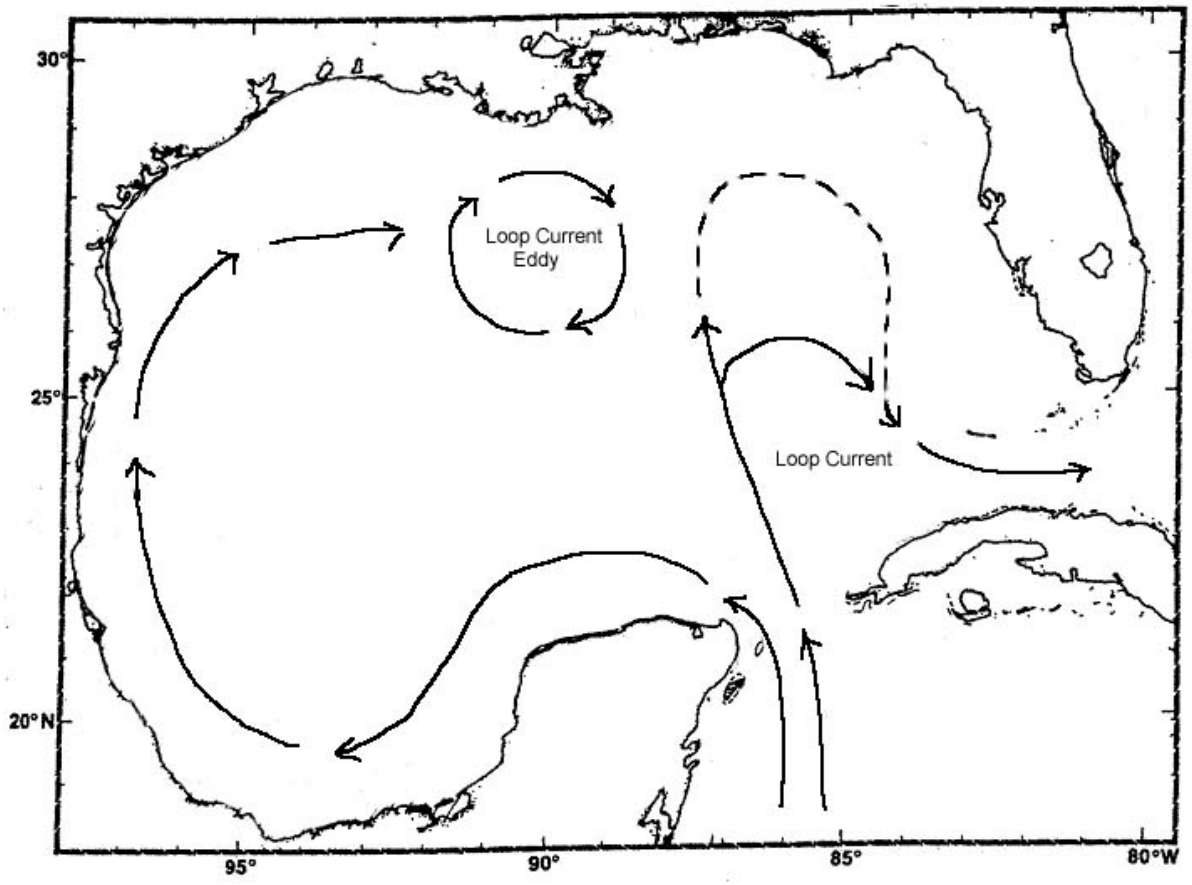


Figure 2. Circulation in the Gulf of Mexico (GOM) is highly variable and is characterized by the Loop Current (LC), Loop Current Eddies (LCEs), and strong coastal jets. The LC is part of the North Atlantic western boundary current that enters the Gulf through the Yucatan Channel. It extends mostly northward before exiting through the Florida Straights. Generally a loop forms in the eastern Gulf and breaks off creating an eddy. Eddies consist of a core of warm water that rotates clockwise as it moves west across the Gulf. Another current occurs along the coast of Mexico. This current extends northward before traveling across the continental shelf in the direction of west to east.

The FGB are located on the outer shelf. The surrounding, semi-diurnal currents rotate clockwise (Lugo-Fernandez, 1998) and are influenced by a variety of processes such as mesoscale deep-water processes and LC rings (Lugo-Fernandez et al., 2001). Flow in this region is predominantly west to east at mean speeds of approximately 8 cm s^{-1} near the surface, decreasing to 1 cm s^{-1} near the bottom (Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001). Mean currents at 60 m over EFGB tend to flow upshelf, while the near bottom currents tend to move offshore (Lugo-Fernandez, 1998). During spawning (following the August full moon), the current along the shelf edge flows mostly eastward, creating a net eastward transport of water as well as possibly larvae (Lugo-Fernandez, 1998).

Warm LC rings are anticyclonic and move westward (Vidal et al., 1992; Berger et al., 1996; Sturges and Leben, 2000; Walker, 2001). The rings along with secondary eddies (cyclonic and anti-cyclonic) are factors that significantly affect and drive circulation near the FGB (Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001; Ohlmann et al., 2001). The average lifespan of LCE is approximately 1 year (Huh and Schaudt, 1990; Vidal et al., 1992; Sturges, 1993; Walker, 2001). The diameters of LC rings range generally from 200-400 km, with surface speeds of 50-75 cm s^{-1} (Huh and Schaudt, 1990; Berger et al., 1996; Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001). Movement of the rings is predominantly to the southwest at 2-6 km/d (Berger et al., 1996; Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001; Ohlmann et al., 2001; Walker, 2001). Smaller, secondary eddies (cold core eddies), often associated with warm core eddies, typically have a life-span of approximately 6 months with diameters ranging from 30-150 km (Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001; Walker, 2001). The separation process of LC rings is a gradual one, requiring up to several months or more (Sturges and Leben, 2000). Circulation associated with eddies in the western and northwestern GOM is thought to be the

main driving force of surface current variability on the outer continental shelf (Walker, 2001). These eddies induce frequent and significant cross-shelf exchange of water with deeper Gulf waters, helping to regulate temperature and salinity, and also influencing larval dispersal (Lugo-Fernanadez, 1998).

Genetic Techniques

There are many different molecular techniques available today to study genetic variability in populations. The choice of which technique to select is dependent upon the application (Vos et al., 1995). Each different molecular technique has its own strengths and weaknesses in methodological aspects, such as the technical expertise available, cost of equipment and consumables, time needed for development, and resolution needed for particular ecological and evolutionary research questions (de Bruin et al., 2003). A molecular marker must have the correct sensitivity for the question, and usually there is a trade-off between the practicality and accuracy of the genetic markers (Sunnucks, 2000).

Allozymes

Allozymes are variant forms of an enzyme that are encoded by different alleles at a specific gene locus. They can be separated on a gel by the use of an electric field, depending on their charge, size, and shape (de Bruin et al., 2003). As a result of the catalytic activity of the enzyme, the locations of different allozymes are revealed through the visible bands produced. Most of the allozymes exhibit co-dominant Mendelian inheritance (Hellberg et al., 2002).

The advantage of using allozymes is that they are easy to develop, and the technique does not require expensive laboratory equipment; however, it is a low-resolution technique (de Bruin et al., 2003). Allozymes only reveal a limited portion of the total variation in the DNA sequence at the encoding locus. The technique requires active enzymes, so samples must be kept alive or

frozen (Hellberg et al., 2002). Since allozymes evolve at a slower rate than mitochondrial DNA and nuclear DNA, they rarely provide enough resolution to assess gene flow levels between populations (Bossart and Prowell, 1998). Another problem with such low resolution in genetic and phenotypic traits is that morphological and physiological traits are not always linked to enzyme banding patterns (de Bruin et al., 2003).

Random Amplified Polymorphic DNA (RAPD)

RAPD is a multi-locus technique, which was first described in 1990. It allows large numbers of markers to be assayed relatively inexpensively using the Polymerase Chain Reaction (PCR; Hill et al., 1996). It is based on the assumption that RAPD loci are dominant Mendelian markers, where the allele amplifies (positive allele) or not (null allele; Hill et al., 1996; Ajmone-Marsan et al., 1997; Hurme and Savolainen, 1999). With RAPDs, short primers, 8 to 10 base pairs in length, are chosen at random (Lin et al., 1996; de Bruin et al., 2003; van Marle-Koster and Nel, 2003). The primers serve as both forward and reverse primers, and the fragments are separated on agarose or polyacrylamide gel-electrophoresis. No prior knowledge of any specific primer sites in the genome of the organism is required. The polymorphisms, which emerge due to variation in the primer annealing sites, are detected by the presence or absence of bands at a particular site (de Bruin et al., 2003).

The advantages of using RAPDs are it requires only a small amount of DNA (Lin et al., 1996; Hill et al., 1996; Ajmone-Marsan et al., 1997; van Marle-Koster and Nel, 2003), and results are easily generated and rapidly analyzed (Lin et al., 1996). It is also an inexpensive (Hill et al., 1996; van Marle-Koster and Nel, 2003) and sensitive technique that reveals a great deal of genetic information, which can be applied to identify specific markers for many kinds of population studies (de Bruin et al., 2003). The main disadvantage of using RAPDs, however, is

its lack of reproducibility, which is dependent upon the annealing of 8 to 10 base pairs, arbitrary sequences of the primers (Lin et al., 1996; Hill et al., 1996; Ajmone-Marsan et al., 1997; Hurme and Savolainen, 1999; van Marle-Koster and Nel, 2003; de Bruin et al., 2003). Reproducibility is also affected by subtle differences in Mg^{++} concentration, dNTP concentration, cycling parameters, and other conditions that considerably affect performance of the PCR reaction (Lin et al., 1996).

RAPD markers have been used in various studies in order to determine genetic variation and relationships among species and populations, genome mapping, phylogenetic description of taxa and subsequent phylogenetic analysis. These markers have been used in numerous genetic studies of plants (Scots pine: Hurme and Savolainen, 1999; apple: Royo and Itoiz, 2004; olive: Wu et al., 2004; Belaj et al., 2004; cauliflower: Astarini et al., 2004) and animals (cattle: Parmar et al., 2004; Bhattacharya et al., 2004; mitten crabs: Lu et al., 2000) because of their abundance as potential markers.

Microsatellites or Short Sequence Repeats (SSRs)

Microsatellites are known as simple sequence repeats (SSRs), short tandem repeats (STRs), or simple sequence length polymorphisms (SSLPs; Robinson and Harris, 1999) that are usually 2 to 10 base pairs in length (Robinson and Harris, 1999; Hellberg et al., 2002; van Marle-Koster and Nel, 2003; de Bruin et al., 2003), for example, $(CA)_{10}$ or $(GC)_{15}$. They are codominant, PCR based markers, allowing heterozygotes to be identified (Hill et al., 1996; Robinson and Harris, 1999; de Bruin et al., 2003). Due to their high mutation rate, microsatellites are often variable enough to discriminate at the individual level (Lucchini, 2003). Repeats tend to occur in non-coding regions of the DNA (de Bruin et al., 2003). Variations result from differences in the number or repeat units, and believed to be caused by errors in the

DNA replication. During replication, the DNA polymerase "slips" when copying the repeat region causing a change in the number of repeats. Screening for microsatellites involves the digestion of genomic DNA, cloning of fragments into vectors, hybridization with probe DNA for positive clones, sequencing cloned DNA, microsatellite primer design, and PCR of fragments containing microsatellite loci (de Bruin et al., 2003).

The advantage of using microsatellites is that it is a very informative technique, which is capable of following the segregation at multi-allelic genetic loci in different crosses (Ajmone-Marsan et al., 1997; Belaj et al., 2004). Being a single-locus marker, microsatellites are more flexible and informative, since they can be analyzed as alleles with frequencies and gene genealogies; however, they are not directly comparable to AFLPs (Robinson and Harris, 1999; Sunnucks, 2000). The disadvantages of using microsatellites are it is expensive, labor-intensive (Hill et al., 1996; Ajmone-Marsan et al., 1997; de Bruin et al., 2003), and requires a high degree of characterization of the target genome. If useful locus-specific primers have not been designed previously, then it is necessary to screen an organism for microsatellites, a complex process that may yield only a small number of potential microsatellite loci (Hill et al., 1996; Robinson and Harris, 1999).

Restriction Fragment Length Polymorphism (RFLP)

RFLP is a molecular technique that involves the use of PCR (Xiao et al., 1999; Meadus et al., 2002). The PCR-RFLP technique amplifies fragments approximately 1000 base pairs in length, that are separated on a gel and visualized by ethidium bromide staining. Unlike the early RFLP protocol, the PCR-RFLP method is much simpler and does not involve the use of Southern blots or probes. The problem with using RFLPs is that they provide less variation than AFLPs. The RFLP technique has been used for placing DNA sequences of known function on genetic

linkage maps (Lin et al., 1996) and for mapping the genomes of many agronomic crops such as tomato, corn, rice, and soybean (de Bruin et al., 2003).

Amplified Fragment Length Polymorphism (AFLP)

AFLPs are the chosen molecular genetic technique for this study. AFLP is a multilocus, DNA-fingerprinting technique (Sunnucks, 2000; Mock et al., 2001) that detects polymorphisms of genetic restriction fragments by PCR amplification (Vos et al., 1995; Mueller and Wolfenbarger, 1999; van Marle-Koster and Nel, 2003). This procedure simultaneously screens many different DNA regions distributed throughout the genome (Muller and Wolfenbarger, 1999). It identifies a greater number of polymorphic DNA markers than any other PCR based detection system (Ajmone-Marsan et al., 1997; Savelkoul et al., 1999), resulting in a powerful technique in terms of its ability to identify a large number of polymorphic bands (Fanizza et al., 2003; Li et al., 2004). AFLPs are dominant markers that are usually detected as the presence or absence of an amplified restriction fragment (0 = band absence, 1 = band presence; Hill et al., 1996; Robinson and Harris, 1999; Mock et al, 2001; de Bruin et al, 2003).

Procedure

The AFLP procedure consists of three main steps. The first step is the digestion of genomic DNA with two restriction enzymes (Vos et al., 1995; Hill et al., 1996; van Marle-Koster and Nel, 2003; de Bruin et al., 2003). Enzymes used in the digest consist of a rare cutter (such as EcoRI) and a frequent cutter (such as MseI or TaqI) restriction endonuclease (Ajmone-Marsan et al., 1997). The frequent cutter ensures the generation of small fragments while the rare cutter limits the number of fragments (Vos et al., 1995; van Marle-Koster and Nel, 2003). To the end of the DNA, adapters of known sequence are attached (Hill et al., 1996), determined by the sequence of AFLP primers (van Marle-Koster and Nel, 2003).

Restriction fragments are then amplified using a pair of primers that are complementary to the adapter sequence and the remains of the restriction site plus up to four random nucleotides at the 3′ end (Hill et al., 1996; Ajmone-Marsan et al., 1997). Primers consist of three main parts: (1) a core sequence, (2) an enzyme specific sequence (ENZ) or 5′ part corresponding to the adapter, and (3) a selective extension (EXT) or 3′ selective nucleotide (Vos et al., 1995). By using primers of defined sequences, the desired DNA fragments are selectively amplified resulting in highly reproducible DNA fragment patterns using standard PCR conditions (Lin et al., 1996).

DNA amplification involves two steps: pre-amplification and amplification. Pre-amplification is performed with two primers having a single selective nucleotide. The reaction mixture is then diluted 10-fold and used as a template for the second amplification reaction (Vos et al., 1995). PCR products are subsequently separated on a polyacrylamide gel according to their size fractionation in a process called gel electrophoresis (Vos et al., 1995; Hill et al., 1996; de Bruin et al., 2003; Li et al., 2004), producing a pattern of 40-200 bands (Savelkoul et al., 1999). The number of bands produced is determined by the specificity of restriction enzymes used to digest the genomic DNA, number and choice of selective nucleotides at the 3′ end of the restriction enzyme, and size and complexity of the genome being analyzed (Lin et al., 1996). Banding patterns obtained are polymorphic due to mutations in the restriction sites, sequences which are adjacent to the restriction sites and complementary to the selective primer extensions, and insertions or deletions associated with the amplified fragment (Savelkoul et al., 1999). The procedure can be manipulated to suit specific applications through the selection of restriction enzymes and design of the PCR primers (van Marle-Koster and Nel, 2003).

Advantages of AFLP

There are many advantages of using AFLPs over other molecular techniques. One advantage is AFLPs produce a large number of polymorphic markers rapidly with fairly simple laboratory work (Lin et al., 1996; Mueller and Wolfenbarger, 1999; Robinson and Harris, 1999; Lucchini, 2003), requiring only a short amount of time to assay large numbers of DNA loci (Hill et al., 1996). The ability to produce multi-locus fingerprints in a single analysis reduces the cost and increases the possibility of detecting polymorphisms (Mickett et al., 2003). Another advantage is no previous known sequence information is required for AFLPs (Vos et al., 1995; Li et al., 2004), making it useful with species for which there is no establishment of polymorphic markers in relation to population identity as well as when there is limited sequence information (Mickett et al., 2003). This is quite different from RFLPs and SSRs that need a high degree of characterization of the target genome (Robinson and Harris, 1999). Another advantage is that the amplification is performed under conditions of high selectivity, making them reliable and highly reproducible, again setting them apart from RAPDs (Lin et al., 1996; Robinson and Harris, 1999; de Bruin et al., 2003; Lucchini, 2003; Van Marle-Koster and Nel, 2003). Since the detection of AFLP fragments does not depend on hybridization, partial digestion, and faint patterns, which are sources of irreproducibility with RFLPs, errors can be easily avoided (Savelkoul et al., 1999). In addition, only a small amount of DNA is required for analysis (Ajmone-Marsan et al., 1997; Mueller and Wolfenbarger, 1999; Savelkoul et al., 1999) allowing minute tissue samples of small organisms to be examined (Mueller and Wolfenbarger, 1999). This makes the technique ideal for examining coral spat and juvenile and adult corals. Compared to RAPDs and RFLPs, AFLPs detect more point mutations per reaction (Li et al.,

2004), and with different combinations of relatively small number of primers, an unlimited number of loci can be assayed (Hill et al., 1996; Mueller and Wolfenbarger, 1999).

Disadvantages

One disadvantage of using AFLPs is their dominant nature (Mueller and Wolfenbarger, 1999; Sunnucks, 2000). DNA fragments are scored only as presence or absence of a band, meaning homozygotes cannot be distinguished from heterozygotes (Sunnucks, 2000; de Bruin et al., 2003). Due to their dominance, more individuals and loci have to be sampled to reveal the same genetic information obtained by other techniques that produce codominant markers (de Bruin et al., 2003). Another weakness of being a multi-locus technique is a portion of the variation they detect may not be derived from the target organism (Sunnucks, 2000). Fortunately, this potential contamination problem has been overcome for corals. Brazeau et al., (submitted) have identified primers specific to zooxanthellae, endosymbionts of corals, which can detect the presence of zooxanthella DNA down to 5-10 picogram levels. In addition, there is no way to assess the homology of the missing bands, and bands of a particular size may occur from different regions of the genome (Robinson and Harris, 1999).

Uses

AFLP is highly reliable for measuring genetic diversity and determining genetic relationships within and among populations and species (Hill et al., 1996; Mueller and Wolfenbarger, 1999; Robinson and Harris, 1999; Mickett et al., 2003). Its ability to distinguish individuals in a population makes AFLPs suitable for paternity analysis, gene flow and dispersal, introgression, hybridization (Mueller and Wolfenbarger, 1999; Robinson and Harris, 1999), and mating frequency (Mueller and Wolfenbarger, 1999). AFLPs have also been used to construct high diversity genetic maps of genomes or genome segments (Vos et al., 1995; Robinson and

Harris, 1999) and in numerous biodiversity and management studies (Lucchini, 2003). Some examples include: reintroduction of Gould's turkey (Mock et al., 2001), aquaculture studies of channel catfish (Mickett et al., 2003) and prawns (Li et al., 2004), cattle and livestock (Ajmone-Marsan et al., 1997; van Marle-Koster and Nel, 2003), and numerous plant studies (Lin et al., 1999; O'Hanlon et al., 1999; Belaj et al., 2004; Fanizza et al., 2003).

Inferring Larval Dispersal from Genetic Data

Many studies have examined connectivity between populations by assessing the frequency of dispersal of propagules of a variety of organisms. Quantifying the dispersal rates in the terrestrial environments has generally involved the use of mark-recapture approaches (Thorrold et al., 2002). This use of mark-recapture, however, is much more difficult in a marine situation for many reasons: (1) organisms often spawn millions of small propagules in single spawning event, making tagging difficult, (2) risk of damage to the eggs and sperm during tagging as a result of handling, and (3) recapture is more difficult and chance of recovery small due to high mortality rates in the early life stages (Thorrold et al., 2002). Today, there are a variety of tagging methods available such as artificial and natural tags that can be used to assess connectivity in populations.

Artificial tags can be used to track larvae. One type of artificial tag is a physical tag that is applied to post-settlement larvae. Another way to track larval dispersal is by visual observation, but the larvae have to be large and visible with the naked eye (Thorrold et al., 2002). In most situations, marine invertebrate larvae are too small to be tracked visually. Another type of artificial tag involves the immersion of larvae in chemicals that become incorporated in the body tissues. Some examples of the chemicals used are fluorescent compounds, elemental tags, and radioisotopes (Thorrold et al., 2002).

Natural tags differ from artificial tags in that the use of a physical tag is completely eliminated. Natural tags rely on differences in environmental conditions to generate population-specific markers (Thorrold et al., 2002). Therefore, every individual from the population is marked. Types of natural tags are environmental tags and genetic markers. Environmental tags rely on marine larvae to experience variations in physical and chemical environments. As a result of these changes, phenotypic variations are often generated in larval form and growth rate (Thorrold et al., 2002). Genetic markers, another type of natural tag, have been used to determine the levels of connectedness between populations based on the genetic similarity of those populations (Hellberg et al., 2002).

In order to estimate gene flow from the distribution of genetic markers in a population, an indirect approach is required (Neigel, 1997). For many years, the standard approach for estimating gene flow has involved the use of allozymes. This involved the use of the Wright's island model of population structure to relate the distribution of allozyme alleles in populations to the product of the effective population size and rate of migration (N_em). This particular approach is referred to as indirect because it infers the magnitude of gene flow from its effects on the distribution of genetic markers (Neigel, 1997). Many studies on larval dispersal of marine organisms have involved the use of allozyme electrophoresis (barnacles: Drouin et al., 2002; corals: Hellberg, 1995; Nishikawa et al., 2002).

With the advancement in genetic techniques, other types of genetic markers have been used. The first DNA based genetic marker system applied to survey genetic variation in natural populations involved the use of animal mitochondrial DNA. The sequence differences were then detected with the use of RFLPs (Neigel, 1997). Another study by Goldson et al. (2002) examined the dispersal capabilities in two bryozoans (*Celleporella hyalina* and *Electra pilosa*)

using RAPDs. The study found that the population structure was dependent on the mode of larval dispersal. *C. hyalina* broods larvae that can swim for $\leq$ 4 hrs. before settling and was found to have a genetic and geographic distance effect; *E. pilosa*, on the other hand, releases larvae that swim for several weeks before competent to settle and did not. In addition, this study showed that *C. hyalina* might be expanding its population at two sites by settling on the fronds of *Fucus serratus* and utilizing them as stepping-stones.

Recently, genetic methods have provided a way to quantify long-distance dispersal. Population assignment tests for individuals based on genetic differentiation among populations have provided the most promising statistical methods used to estimate contemporary long-distance dispersal (He et al., 2004). Assignment tests identify the population to which, statistically, the genotype most likely belongs. When the individual is assigned to a population other than the one from which it was sampled, it is interpreted as evidence of a dispersal event. One major advantage of using assignment methods is populations do not have to be sampled exhaustively (He et al., 2004). A study by He at al. (2004) examined seed dispersal in *Banksia hookeriana*, fire-killed shrub, with the use of AFLPs. This study is one of the first studies using AFLPs to assess the extent of long-distance dispersal in plant populations by population allocation analysis using a new procedure that has been developed for AFLP data called AFLPOP v. 1.0 (Duchesne and Bernatchez, 2002). AFLPs were chosen for this population assignment study because increasing the number of markers (loci) was more critical in generating information content than increasing allelic diversity per locus. Molecular markers, especially the use of AFLPs, provide novel approaches to accurately quantifying distance dispersal in a wide variety of organisms.

MATERIALS AND METHODS

Study Site

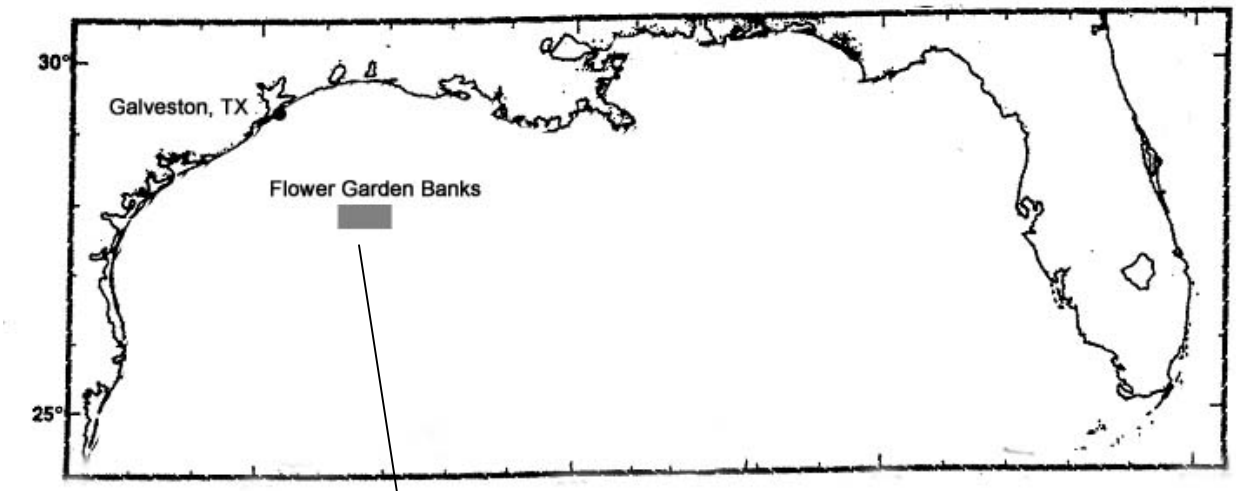
The initial sites surveyed for this study were thirteen oil and gas platforms off the Louisiana and Texas coast. Of the thirteen platforms surveyed, six of them either did not support any coral growth or lacked the target species. Only the seven platforms colonized by the target species will be referred to here (Fig. 3). To simplify discussion, platforms will be referred to by numbers 1 to 7 in the order that they are located from west to east, instead of by platform name (see Table 2). Tissue was collected from coral colonies on each of the seven platforms. Samples were also collected from the East and West FGB, located approximately 180 km southeast of Galveston, Texas, USA. These platforms fell within an elongated ellipse, inclusive of the FGB - extending from 10 to 15 km west of the WFGB to 50 km east of EFGB and 10-15 km north. The sampling regime was skewed to the east because of the known prevailing westerly currents in the region (Sturges, 1993; Oey, 1995; Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001).

Sample Collection

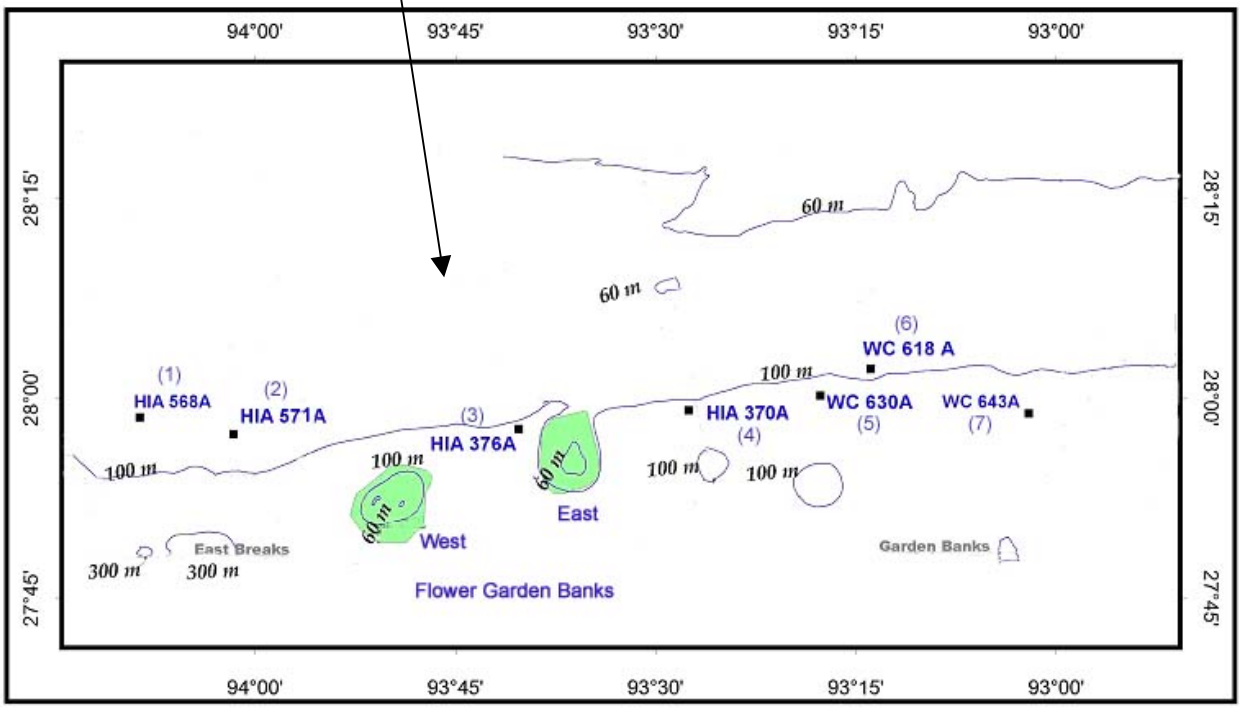
Tissue samples were collected from adult colonies of three different species (*Diploria strigosa*, *Madracis decactis*, and *Montastraea cavernosa*) between 0 and 30 m depth. The number of samples collected per species varied with each platform, depending on its presence and abundance there (Table 3).

Teams of SCUBA divers collected coral samples, approximately 2 cm^2 in size, which were removed with a small hammer and chisel from the growing edge of the colony. Samples were placed into small, sealed plastic freezer bags, containing SED buffer (saturated NaCl, 250 mM EDTA, pH 7.5, 20% DMSO) to preserve the DNA. This buffer allows the samples to be stored at room temperature, eliminating the need for storage in liquid nitrogen (at -196ºC). Bags

were then placed into ice chests containing additional high-salt buffer and returned to the laboratory.



(a)



(b)

Figure 3. (a) Map of the northwestern Gulf of Mexico showing the location of the Flower Garden Banks, approximately 180 km S-SE of Galveston, Texas. (b) Map depicting the study sites including the Flower Garden Banks and the 7 platforms sampled in this study. Numbers 1-7 were assigned to each platform in the order that they occurred from west to east for simplifying platform name and reference location.

Table 2. Details of the seven oil and gas platforms studied, including an assigned platform number by location of west to east; platform name, coded by lease area; MMS reference number; corporate owner at the time of sampling; location, by latitude and longitude; date of deployment; age in years at time of the study; and distance from the perimeter of the Flower Garden Banks in kms.

Platform Reference No., W to E	Platform	MMS No.	Owner	Lat.	Long.	Date of Deployment	Age at Time of Sampling (years)	Distance from FGB perim. (km)
1	HI-A-568A	10133	Samedan	27.9763	94.1439	1979	22	24.46
2	HI-A-571A	10116	W&T Offshore	27.9556	94.0271	1978	23	15.05
3	HI-A-376A	10175	Anadarko	27.9620	93.6709	1981	20	2.01
4	HI-A-370A	10060	Kerr-McGee	27.9855	93.4584	1976	25	10.86
5	WC-630A	22031	Forcenergy	28.0034	93.2941	1977	24	24.14
6	WC-618	23286	Newfield	28.0367	93.2312	1986	15	30.18
7	WC-643A	21757	Texaco	27.9814	93.0342	1975	26	48.68

Table 3. Sample sizes for the three coral species at each of the nine locations examined in this study.

Platform #		Sample Size for Each Species		
W to E	Study Sites	*Madracis decactis*	*Diploria strigosa*	*Montastraea cavernosa*
1	HI-A-568A	8	0	0
2	HI-A-571A	15	0	0
WFGB	West FGB	25	29	27
3	HI-A-376A	20	5	0
EFGB	East FGB	18	23	25
4	HI-A-370A	19	0	0
5	WC-630A	28	4	0
6	WC-618	7	0	0
7	WC-643A	27	3	8
Total		167	64	60

Preparation of Coral Tissue Lysates

In the laboratory, tissue was removed with a razor blade from the coral samples and placed into 1.7 ml labeled centrifuge tubes. Tissue was suspended with SED buffer and stored at room temperature until further analysis. DNA was isolated and purified using the Wizard® SV Genomic DNA Purification System (Promega Corporation, Madison, WI), following manufacturer's instructions for animal tissue (Refer to Appendix). SED buffer was removed prior to this process.

AFLP Protocol

Part A. Genomic DNA digestion and adapter ligation

A restriction-ligation "master mix" was prepared according to the number of samples to be analyzed. The components for one reaction are listed here. The reagents include: 1.1 µl T_4 DNA ligase 10X buffer (30 mM Tris-HCl, pH 7.8 / 10 mM $MgCl_2$ / 10mM dithiothreitol (DTT) / 1mM ATP), 1.1 µl of 0.5 M NaCl, 0.5 µl bovine serum albumin (BSA; 1 mg/ml), 1.0 µl *Mse* I adapters (50 µM), 1.0 µl *Eco*RI adapter (5 µM), 0.25 µl *Mse* I (4U/µl; New England BioLabs,

Beverly, MA), 0.25 µl of *Eco*RI (20U/µL; New England BioLabs), and 0.33 µl of T_4 ligase (3 U/µl; 10 mM Tris-HCl, pH 7.0 / 50mM KCl / 1mM DTT / 0.1 mM EDTA / 50% glycerol) for a total volume of 5.53 µl (Refer to Appendix). Before adding the above adapters to the mix, the adapters were heated to 95ºC for 5 min, cooled to room temperature for 10 min, and centrifuged for 10 s. Sequences for the *Mse* I and *Eco*RI adapters are listed in Table 4. To each new 1.7 ml tube, 5.5 µl of the restriction-ligation mixture plus 5.5 µl of the purified genomic was added, centrifuged for 15 s, and incubated at room temperature overnight. At the end of the restriction-ligation reaction, 189 µl of TE buffer (10 mM Tris-HCl, pH 8.0 / 0.1 mM EDTA) was added (10 fold dilution), serving as the template for the next step, preselective amplification.

Table 4. List of adapters and primers used in the AFLP protocol along with their sequence information. Preselective and selective nucleotides are indicated in bold.

	Name	**Sequence**
Adapters *Eco*RI	*Eco* top strand	5'-CTCGTAGACTGCGTACC
	Eco bottom strand	5'-AATTGGTACGCAGTCTAC
Adapters *Mse*I	*Mse* top strand	5'-GACGATGAGTCCTGAG
	Mse bottom strand	5'-TACTCAGGACTCAT
Preselective primer	*Eco*RI **A**	5'-GACTGCGTACC AATTC **A**
Preselective primer	*Mse*I **C**	5'-GATGAGTCCTGAG TAA **C**
Selective primers (Set 1)	*Eco*RI	5'-GACTGCGTACCAATTC **ACT**
	*Mse*I	5'-GATGAGTCCTGAGTAA **CAG**
Selective primers (Set 2)	*Eco*RI	5'-GACTGCGTACCAATTC **ACC**
	*Mse*I	5'-GATGAGTCCTGAGTAA **CTT**

Part B. Preselective (PS) Amplication

For the next step, a "master mix" was made according to the number of samples to be analyzed. Preselective amplification components for one reaction are listed as the following: 8.1 µl of nuclease-free water, 2.0 µl of 10X PCR buffer (15 mM Mg^{++} in buffer), 0.8 µl of 5 mM dNTP's, 2.0 µl of *Eco*RI PS primer (2.75 µM), 2.0 µl of *Mse*I PS primer (2.75 µM), and 0.1 µl of Thermostable (Taq) DNA polymerase (5U/µl), for a total volume of 15.0 µl (see Appendix). Sequences of the *Eco*RI and *Mse*I PS primers are listed in Table 4. To each 0.5 ml tube, 15 µl of the preselective amplification master mix was added, followed by a drop of mineral oil to prevent the DNA from evaporating during the cycling of temperatures. Next, 5 µl of each of the DNA samples (Digestion-Ligation from Part A) was added to each corresponding tube, vortexed, centrifuged for 15 s, and placed into a Biometra UNO-Thermoblock (Biometra, Göttingen, Germany). Amplification was performed according to the profile below: 2 min initial incubation at 72ºC, followed by 20 cycles of 20 s denaturation at 94ºC, 30 s annealing at 56ºC, and 2 min extension at 72ºC. Last steps were 2 min final extension at 72ºC, and 30 min final incubation at 60ºC. After the cycling was completed, 180 µl of TE buffer was added to each tube, which consisted of the templates for the final step, selective amplification (Part C).

Part C. Selective Amplification

The final step of the AFLP protocol consists of making a selective amplification "master mix" containing the following components (1 reaction): 8.1 µl of nuclease-free water, 2.0 µl of 10X PCR buffer (Mg^{++} in buffer), 0.8 µl of 5mM dNTP's, 2.0 µl of *Eco*RI selective primer (0.46 µM), 2.0 µl of *Mse*I selective primer (2.75 µM), and 0.1 µl of Taq DNA polymerase (5U/µl) for a total volume of 15.0 µl. To each 0.5 ml micro-centrifuge tube, 15 µl of the selective amplification master mix was added, followed by one drop of mineral oil. From the preselective

amplification (Part B), 5 µl of the diluted PCR product was added to each corresponding tube, mixed, and centrifuged for 15 s. Next, they were placed in the thermocycler and the cycle profile was performed as indicated: 2 min initial denaturation at 94ºC, followed by 1 cycle of 20 s denaturation at 94ºC, 30 s annealing at 66ºC, and 2 min extension at 72ºC. Next, there were 9 cycles: 20 s at 94ºC, initial 30 s at 66ºC (reduced 1ºC/cycle), and 2 min at 72ºC. Final cycle consisted of 20 cycles: 20 s at 94ºC, 30 s at 56ºC, and 2 min at 72ºC, followed by a 30 min final incubation at 60ºC.

Polyacrylamide Gel Electrophoresis

The products of the selective PCR were separated on a 5% polyacrylamide (sequencing) gel, following a standard recipe for preparation (see Appendix). Approximately 15 µl of coral DNA was loaded into the wells of the gel, which was run at 55-60 W for 1 h in TEB buffer (pH 8.6; Refer to Appendix). Next, the gels were stained using Silver Sequence^{TM} DNA Sequencing System (Promega Corporation; Refer to Appendix). Banding patterns were analyzed using Kodak Digital Image Analysis software (Eastman Kodak Co. Scientific Imaging Systems; Bonin et al., 2004).

Statistical Analyses

Two statistical analyses were used in this study: Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992), and AFLPOP v. 1.0 (Duchesne and Bernatchez, 2002). AMOVA estimates population differentiation directly from molecular data and tests hypotheses about such differentiation (Excoffier et al., 1992). This method allowed for the use of molecular marker data, which can be nucleotide based, base sequence, or restriction fragment. This program treats any kind of raw data as a Boolean vector p_i. A matrix is created consisting of binary data – 1's and 0's (1 indicating presence and 0 absence). Next, the Euclidean distances

between pairs of vectors are calculated by subtracting the Boolean vector of one haplotype from another (p_j-p_k). The Euclidean distance is a scalar that is equal to the shortest distance between those two points. Next, squared Euclidean distances are calculated for all pairwise arrangements of Boolean vectors, which are arranged in a matrix and partitioned into submatrices corresponding to subdivisions in the population (Excoffier et al., 1992). The sum of squares for various hierarchical levels of the population is calculated using the sums of the diagonals in the matrix and submatrices. The sum of squares can be analyzed in a nested analysis of variance (ANOVA) framework.

The variance components can be used to calculate a series of statistics called phi-statistics (Φ_{ST}), which summarize the degree of differentiation between population divisions. Phi-statistics are analogous to F-statistics and are evaluated using a permutational statistical approach, eliminating the normality assumption that is conventional for analysis of variance, but is not appropriate for molecular data (Excoffier et al., 1992). The phi-statistic can be treated as an indicator of degree of population differentiation. The data are not expected to follow a null distribution, because the molecular data consist of Euclidean distances derived from 1's and 0's. A null distribution is computed by re-sampling the data. AMOVA examines the distances among all alleles and calculates an average. A bootstrap analysis of 1000 iterations was performed for each species of coral, and the data were presented in a matrix. The portion above the diagonal indicates the significance level, while the portion below represents distance measures (Φ_{ST} value). The significance levels (p-values) were corrected using a Bonferroni correction, which is an adjustment used for multiple comparisons (Bonferroni, 1935, 1936). The Bonferroni correction decreases the chance of Type I error, controlling for false positives.

AFLPOP was also used to quantify genetic affinity between individuals and populations in this study (Duchesne and Bernatchez, 2002; He et al., 2004). It is an assignment test that, given a genotype and a set of sampled populations, can statistically determine and identify the population to which the genotype most likely belongs. It uses AFLP data (similar presence/absence data) to calculate log-likelihood values for individuals derived from a reference population, based upon their banding patterns. Next, each individual is allocated to the population showing the highest likelihood for that genotype. Thus, it is a more sensitive program than AMOVA. AFLPOP provides an alternative approach for examining population differentiation and is designed to be used specifically with AFLP data.

AFLPOP Randomization Analyses

The AFLPOP program provides the option of choosing the minimal log-likelihood difference – a threshold – beyond which individuals are assigned to a population. The options were log (0) or log (1). Allocation of the individuals is based on the difference between the highest log-likelihood and the second highest log-likelihood. With Log 0, the program will allocate as soon as there is a highest log-likelihood reached among the populations. By increasing the threshold of the program, it reduces the probability of incorrect allocation. In order to determine the best option, for this threshold, 100 randomized data sets were generated using two *Madracis decactis* populations, drawn from platforms WC-643 and HI-A-370, respectively. The populations used were previously found to be highly significantly different by AMOVA, some of the larger sample sizes in the study (n = 27, 20, respectively). The 100 randomized data sets were run using a minimum log-likelihood difference first of 0 and then of 1. The Log 0 option requires only that the genotype be more likely to occur in one population *vs*. another for assignment. The Log 1 option, however, is much more conservative. Here, the

multilocus genotype must be 10 times more likely to occur in the potential population of assignment than in any other population. If the genotype does not meet this criterion, it is not assigned to any population, indicated here by a "None" category.

In addition, the means and 95% confidence limits of the 100 randomized data sets were calculated using BIOMstat v. 3.2 (Applied Biostatistics, Inc., 1996). Then each of the 100 randomized data sets was compared with the actual data for each platform for both Log 0 and Log 1 using a G-test of independence with Williams' correction (Sokal and Rohlf, 1981), to determine how many of the randomized runs were significantly different from the actual data (BIOMstat, Applied Biostatistics, Inc., 1996).

RESULTS

A total of 291 tissue samples covering three species of coral were examined in this study. Two primer sets, 1A/1C and 1A/4C, were used in the analysis. For *Montastraea cavernosa*, 60 colonies were analyzed yielding a total of 40 polymorphic markers. Primers 1A/1C produced 22 markers while 1A/4C produced 18 polymorphic markers. Sixty-four *Diploria strigosa* colonies were analyzed, producing a total of 31 polymorphic markers, 15 for 1A/1C and 16 for 1A/4C. For *Madracis decactis*, 57 polymorphic markers were obtained from the 167 colonies analyzed. Primer set 1A/1C and 1A/4C produced 39 and 18 polymorphic markers respectively.

AMOVA

Madracis decactis

AMOVA analysis for *Madracis decactis* examined nine populations, Platforms 1-7, and the East and West FGB, using a bootstrap analysis of 1000 iterations. Firstly, the two FGB were found to be homogeneous (Table 5). The *M. decactis* population from Platform 1 (platform furthest west) was significantly different from all others. The population from Platform 2 was significantly different from Platforms 1, 3, 5, 6, and 7. The WFGB was genetically homogeneous with all populations except with Platform 1. Platform 3 (located between the two banks) was significantly different from Platforms 1, 2, 4, and 6. The EFGB population indicated a significant difference between the platform furthest west and the two platforms furthest east. Platform 4 exhibited a similar pattern to that of Platform 2, which was found to be significantly different from Platforms 1, 3, 6, and 7. The *M. decactis* population from Platform 5 was found to be significantly different from the two platforms furthest west and Platform 6. Similar to Platform 1, Platform 6 was significantly different from all populations except for the WFGB. Platform 7 was found to be genetically homogeneous with the WFGB and Platforms 3 and 5.

Table 5. *Madracis decactis*: Assessment of genetic differentiation among populations of the coral *Madracis decactis* using Analysis of Molecular Variance (AMOVA). Significance based upon analysis of 1000 iterations (upper diagonal). P-values were adjusted using Bonferroni correction. Φ_{ST} values shown on the lower diagonal.

	Reference Population								
Compared to ↓	**(1) HI-A-568**	**(2) HI-A-571**	**W-FGB**	**(3) HI-A-376**	**E-FGB**	**(4) HI-A-370**	**(5) WC-630**	**(6) WC-618**	**(7) WC-643**
HI-A-568		**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**	**P < 0.001**
HI-A-571	0.1285		1.00	**P < 0.001**	1.00	1.00	**P < 0.001**	**P < 0.001**	**P < 0.001**
W-FGB	0.0763	0.0421		1.00	1.00	1.00	1.00	1.00	1.00
HI-A-376	0.0740	0.0730	0.0129		1.00	**P < 0.001**	1.00	**P < 0.001**	1.00
E-FGB	0.0828	0.0250	-0.0057	0.0116		1.00	1.00	**P < 0.001**	**P < 0.001**
HI-A-370	0.1133	0.0035	0.0189	0.0348	0.0059		1.00	**P < 0.001**	**P < 0.001**
WC-630	0.0674	0.0392	0.0176	0.0198	0.0012	0.0178		**P < 0.001**	0.972
WC-618	0.0650	0.1140	0.0771	0.0967	0.1060	0.0951	0.1097		**P < 0.001**
WC-643	0.0987	0.1266	0.0476	0.0148	0.0485	0.0858	0.0300	0.1385	

Diploria strigosa

For *D. strigosa*, five populations were analyzed: East and West FGB, and Platforms 3, 5, and 7. Again, the two FGB were found to be genetically homogeneous (Table 6). AMOVA analysis demonstrated that the WFGB was genetically homogeneous with all populations except for Platform 3. It was also shown that *D. strigosa* population from Platform 5 was highly significantly different from the WFGB and Platform 5 ($p < 0.001$). In addition, Platform 7 was significantly different from Platform 5 ($p < 0.001$). The EFGB was homogeneous with all other populations.

Table 6. *Diploria strigosa*: Assessment of genetic differentiation among populations of the coral *Diploria strigosa* using Analysis of Molecular Variance (AMOVA). Significance based upon analysis of 1000 iterations (upper diagonal). P-values were adjusted using Bonferroni correction. Φ_{ST} values shown on the lower diagonal.

	Reference Population				
Compared to ↓	**W-FGB**	**(3) HI-A-376**	**E-FGB**	**(5) WC-630**	**(7) WC-643**
W-FGB		**P < 0.001**	1.00	1.00	0.460
HI-A-376	0.0629		1.00	**P < 0.001**	1.00
E-FGB	0.0127	0.0508		1.00	1.00
WC-630	0.0222	0.1657	0.0183		**P < 0.001**
WC-643	0.0686	0.0121	0.0380	0.1975	

Montastraea cavernosa

Three populations, East and West FGB, and Platform 7, were analyzed for *M. cavernosa*. Bootstrapping analysis using 1000 iterations showed significant genetic differentiation among two of the three groups (Table 7), with the exception of the WFGB and Platform 7. The AMOVA analysis indicated that the two banks were highly significantly different from one another ($p < 0.001$). Platform 7 was significantly different from the EFGB.

Table 7. *Montastraea cavernosa*: Assessment of genetic differentiation among populations of the coral *Montastraea cavernosa* using Analysis of Molecular Variance (AMOVA). Significance based upon analysis of 1000 iterations (upper diagonal). P-values were adjusted using Bonferroni correction. Φ_{ST} values shown on the lower diagonal.

	Reference Population		
Compared to ↓	**W-FGB**	**E-FGB**	**(7) WC-643**
W-FGB		**P < 0.001**	0.995
E-FGB	0.0881		**P < 0.001**
WC-643	0.0101	0.1154	

AFLPOP

Randomization Analyses

To test the various properties of the AFLPOP statistical analysis, the original highly significantly different data sets derived from *Madracis decactis* of platforms WC-643 and HI-A-370 were merged, randomized, and re-assigned to their original "labels". The new randomized populations were then analyzed by AFLPOP using a minimum log-likelihood difference of 0. This was repeated 100 times. Means and 95% confidence limits of derived values for the different runs for each population were calculated and are listed in Table 8. The "new home reference" indicates the percentage of randomized colonies that were allocated back to their newly defined population of origin as well as those allocated to the other population. Each platform was used as a reference population, respectively. Note the high level of self-allocation using a log-likelihood difference option of "0".

Each of the newly generated, randomized *Madracis decactis* populations was compared against the actual original data using a G-test of independence with Williams' correction. A significant difference ($p < 0.05$) occurred between the average frequencies of the randomized run results versus the actual data for platform WC-643 (Table 9a). Each individual randomized test

run result was then compared to the actual data to determine how many of the 100 randomized runs were significantly different from the original source data. Of the 100 runs for platform WC-643 using a minimum log-likelihood difference of zero, 56 ratios derived from the randomized data were significantly different from the ratio of the actual source data; 44 ratios were not. When considering platform HI-A-370, 52 ratios derived from the randomized data were significantly different from the ratio of the actual source data; 48 ratios were not (Table 9b).

Table 8. Average percentages and 95% confidence limits of 100 different runs of AFLPOP using a minimum log-likelihood difference of zero. Data derived from *Madracis decactis* populations on platforms WC-643 and HI-A-370, which were merged and randomly reassigned to these labels prior to analysis. Process repeated 100 times.

***Madracis decactis* (Log 0; Randomized Data)**

		New Home Reference	
Allocated to ↓		**WC-643**	**HI-A-370**
WC-643	$\bar{Y}$	87.7	11.9
	95% c.l.'s	87.0 - 88.4	11.2 - 12.6
HI-A-370	$\bar{Y}$	12.3	88.1
	95% c.l.'s	11.6 -13.0	87.4 - 88.8

Using the same source data sets of *Madracis decactis* the results were quite different when a minimal log-likelihood difference of one was used. Once again by using Log 1, the multi-locus genotype has to be 10 times more likely to occur in that population than any other population in order to be assigned there. The means and 95% confidence limits of the different runs for each population using this option are listed in Table 10. The "new home reference" indicates the percentage of colonies that were allocated back to its population of origin as well as those allocated to the other population.

Table 9. (a) Colonies of *Madracis decactis* from platform WC-643. The percentage of colonies allocated back to the home reference population (population of origin) are shown for both the actual data and the average run results of 100 randomizations. The actual data and average of runs of randomized data were analyzed in AFLPOP using a minimum log-likelihood difference of zero. Log 0 indicates the minimal log-likelihood difference to allocate a specimen, requiring only that the genotype be more likely to occur in one population relative to the others.
(b) Similar AFLPOP analysis, but considering the *Madracis decactis* population from platform HI-A-370.

(a)

Home Reference Population
WC-643

Allocated to ↓	**Source Data**	**Average Run Results of Data Randomized from the Home Ref. Pop.**
WC-643	98.5%	87.7%
HI-A-370	1.5%	12.3% (n=100)

(b)

New Home Reference Population
HI-A-370

Allocated to ↓	**Source Data**	**Average Run Results of Data Randomized from the Home Ref. Pop.**
WC-643	2.3%	11.9%
HI-A-370	97.7%	88.1% (n=100)

The average of randomized data analysis results for all runs were compared with the actual data using a G-test of independence with Williams' correction. This was done for each platform as well. A highly significant difference occurred between the average run of all randomized results versus the actual data for platform WC-643 ($p < 0.001$; Table 11a). Each individual randomized test run result was then compared with the actual data to determine how

many of the 100 runs were significantly different. In this case, using the Log 1 option, of the 100 runs for platform WC-643, 94 ratios derived from the randomized data were significantly different from the ratio of the actual source data; 6 ratios were not. Similar to platform WC-643, a highly significant difference was found between the average of runs or randomized data and the actual data for the *Madracis decactis* population located on platform HI-A-370 (Table 11b). Of the 100 runs for HI-A-370, 95 ratios derived from the randomized data were significantly different from the ratio of the actual source data; 5 ratios were not.

The number of significant differences between source data and randomized run results was much higher using a Log 1 option than a Log 0. Since Log 1 was determined to be the more conservative and reliable option for testing for differences between populations, all further analyses were performed with this option.

Table 10. Average percentages with 95% confidence limits of 100 different runs of AFLPOP using a minimum log-likelihood difference of one. Derived from randomized data drawn from *Madracis decactis* populations on platforms WC-643 and HI-A-370. Data from these two highly significantly different populations were merged and then randomly assigned back to their "New Home Reference" sites. "None" indicates the percentage of colonies assigned to either "New Home Reference" population.

***Madracis decactis* (Log 1; Randomized Data)**

		New Home Reference	
Allocated to ↓		**WC-643**	**HI-A-370**
WC-643	$\bar{Y}$	57.9	1.4
	95% c.l.'s	55.42 – 60.31	1.32 – 1.46
HI-A-370	$\bar{Y}$	1.5	56.8
	95% c.l.'s	1.42 – 1.54	54.43 – 59.26
None	$\bar{Y}$	40.9	42.0
	95% c.l.'s	38.37 – 43.52	39.50 – 44.43

Table 11. (a) *Madracis decactis* from platform WC-643. Percentage shown relate to percentage of colonies allocated back to the home reference population (population of origin) for both the original source data and the average of all randomized run results. The source data and average randomized run results were analyzed by AFLPOP using a minimum log-likelihood difference of one. With Log 1, the multilocus genotype must be 10 times more likely to occur in that population than in any other to be assigned. (b) AFLPOP analysis using a minimum log-likelihood difference of one for the *Madracis decactis* population on platform HI-A-370, indicating the percentage of colonies allocated back to the home reference population for both the actual data and the average of runs of randomized data.

(a)

Home Reference Population
WC-643

Allocated to ↓	**Source Data**	**Average Run Results of Data Randomized from the Home Ref. Pop.**	
WC-643	93.1%	57.9%	
HI-A-370	0.3%	1.5%	
None	6.6%	40.9%	(n=100)

(b)

New Home Reference Population
HI-A-370

Allocated to ↓	**Source Data**	**Average Run Results of Data Randomized from the Home Ref. Pop.**	
WC-643	0.5%	1.4%	
HI-A-370	93.3%	56.8%	
None	6.2%	42.0%	(n=100)

Madracis decactis

With respect to *Madracis decactis*, the AFLPOP analysis indicated that populations from Platforms 1 and 6 had the two highest percentages (94%, 97%, respectively) of re-allocation back to the population of origin (Table 12). Both the East and West FGB showed two of the

lowest percentages of self-allocation of *M. decactis* colonies (26%, 32%, respectively), and the highest percentages of non-assignment. With respect to Platforms 3 and 4, a similar pattern was exhibited by both platforms, which had an average of 38% of the colonies allocated back to itself with approximately 59% of the colonies not assigned to any population. Platform 5, similar to the West FGB, allocated an average of 31% of the *M. decactis* colonies back to itself with 66% of the colonies not assigned to any population. The population from Platform 7 allocated only half of its colonies back to its population of origin, while the other half were not assigned to any population.

Diploria strigosa

AFLPOP analysis, executed using a log 1 option, indicated that the majority ($\geq$ 83%) of the *Diploria strigosa* colonies analyzed were allocated back to their reference population (Table 13). The East & West FGB, however, showed the lowest averages of self-allocation (33%, 34%, respectively) with approximately 64% of the *D. strigosa* colonies not assigned to any population. Platform 3 had a high percentage (83%) of colonies allocated back to itself. Two populations, Platforms 5 and 7, well to the east, had 100% allocation of colonies back to their population of origin (reference population).

Montastraea cavernosa

AFLPOP analyses showed that $\geq$ 68% of the *Montastraea cavernosa* colonies were allocated back to their reference populations (Table 14). Unlike *Madracis decactis* and *D. strigosa*, both banks had a high allocation of colonies back to their population of origin. The East FGB had the highest percentage of self-allocation (85.2%). Platform 7 had 74% of its colonies allocated back to itself with 24.7% not assigned to any population.

Table 12. *Madracis decactis*: Assignment of samples from adult colonies of *Madracis decactis* to their reference home population *vs.* other sites. Data analyzed via AFLPOP of band frequencies, using a minimum log-likelihood difference of one. Adult assignments based upon simulation of 500 randomly chosen individuals for the adult samples. Levels of allocation back to reference populations (populations of origin) shown on the diagonal in bold. Pairwise comparisons shown.

	Reference Population								
Allocated to ↓	**(1) HI-A-568**	**(2) HI-A-571**	**W-FGB**	**(3) HI-A-376**	**E-FGB**	**(4) HI-A-370**	**(5) WC-630**	**(6) WC-618**	**(7) WC-643**
HI-A-568	**94.3%**	0.0%	0.0%	0.2%	0.0%	0.0%	0.0%	0.0%	0.1%
HI-A-571	0.0%	**62.4%**	0.2%	0.1%	0.2%	1.2%	0.3%	0.0%	0.0%
W-FGB	0.0%	0.3%	**32.2%**	0.3%	0.3%	0.4%	0.6%	0.0%	0.3%
HI-A-376	0.0%	0.1%	0.4%	**38.5%**	0.3%	0.4%	0.4%	0.0%	0.4%
E-FGB	0.0%	0.2%	0.8%	0.3%	**26.2%**	0.5%	0.5%	0.0%	0.1%
HI-A-370	0.0%	0.5%	0.6%	0.4%	0.4%	**37.8%**	0.6%	0.0%	0.0%
WC-630	0.0%	0.2%	0.3%	0.2%	0.4%	0.4%	**30.9%**	0.0%	0.2%
WC-618	0.0%	0.0%	0.2%	0.1%	0.2%	0.0%	0.0%	**97.1%**	0.0%
WC-643	0.1%	0.0%	0.3%	1.2%	0.4%	0.2%	1.0%	0.0%	**50.4%**
None	5.6%	36.3%	65.0%	58.7%	71.8%	59.1%	65.7%	2.9%	48.5%
Totals	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 13. *Diploria strigosa*: Assignment of samples from adult colonies of *Diploria strigosa* to their reference home population *vs*. other sites. Data analyzed via AFLPOP of band frequencies, using a minimum log-likelihood difference of one. Adult assignments based upon simulation of 500 randomly chosen individuals for the adult samples. Levels of allocation back to reference populations (populations of origin) shown on the diagonal in bold. Pairwise comparisons shown.

	Reference Population				
		(3)		**(5)**	**(7)**
Allocated to ↓	**W-FGB**	**HI-A-376**	**E-FGB**	**WC-630**	**WC-643**
W-FGB	**34.1%**	0.1%	1.2%	0.0%	0.0%
HI-A-376	0.4%	**83.3%**	0.9%	0.0%	0.0%
E-FGB	1.4%	0.1%	**32.7%**	0.0%	0.0%
WC-630	0.2%	0.0%	0.1%	**100.0%**	0.0%
WC-643	0.1%	0.0%	0.2%	0.0%	**99.8%**
None	63.8%	16.5%	64.9%	0.0%	0.2%
Totals	100.0%	100.0%	100.0%	100.0%	100.0%

Table 14. *Montastraea cavernosa*: Assignment of samples from adult colonies of *Montastraea cavernosa* to their reference home population *vs*. other sites. Data analyzed via AFLPOP of band frequencies, using a minimum log-likelihood difference of one. Adult assignments based upon simulation of 500 randomly chosen individuals for the adult samples. Levels of allocation back to reference populations (populations of origin) shown on the diagonal in bold. Pairwise comparisons shown.

	Reference Population		
			(7)
Allocated to ↓	**W-FGB**	**E-FGB**	**WC-643**
W-FGB	**68.2%**	0.2%	0.9%
E-FGB	1.5%	**85.2%**	0.2%
WC-643	1.2%	0.1%	**74.2%**
None	29.1%	14.5%	24.7%
Totals	100.0%	100.0%	100.0%

DISCUSSION

The AFLP technique yielded a total of 57 markers for *Madracis decactis*, 31 for *Diploria strigosa*, and 40 for *Montastraea cavernosa*. One question that commonly arises in this type of work with corals is the possibility of zooxanthellar contamination in the coral DNA. This issue, however, has been addressed in previous studies and was addressed through the use of primers that specifically amplify zooxanthellar DNA to confirm that contamination was not a factor here and was not contributing AFLP bands to the adult coral data (Lopez et al., 1999; Brazeau et al., submitted).

In *Madracis decactis*, the AMOVA analysis showed that the East and West FGB were not genetically distinct. This suggests that the two banks are interconnected to one another and that a cross-exchange of genetic information via larval dispersal has been occurring. This is not surprising, since these reefs have co-existed in close proximity (<12 km) for approximately 10,000-15,000 years. The analysis also indicated that Platform 1 (platform furthest west, approximately 24.5 km from the nearest FGB perimeter) was significantly different from all other populations. This may be due to Founder effect or possible influence by some other outside larval source. Founder effect occurs when a few pioneer individuals of an original population start a new population. As a result of a single random event at some time in the past, a batch of larvae in the water column with a given genetic signature could have encountered this particular platform and settled. Due to small sample size (in this case 8), the new population – (1) could have a reduced or a much different genetic signature than the original population; or (2) may be the result of a non-random sample of the genes in the original population. An outside larval source may also be a contributing factor. The nearest probable larval source is the Lobos-Tuxpan Reef System, located 13 km off the coast of Cabo Rojo (approximately 640 km SW of

the FGB), or the Campeche Bank Reefs, located 181 km off the Yucatan Peninsula. Similarities have been found to exist between the FGB and Campeche reefs, and the FGB is believed to be an extension of communities that occur in the southern GOM (Rezak et al., 1983b; Bright et al., 1984). Colonization of larvae from an outside source at this location may be the result of: (1) random settlement event due to local current regimes at the time or even from natural disturbances such as hurricanes or storms; or (2) the utilization of available space by coral larvae, encountered by chance. In order to determine the probable origin of the *Madracis* colonies on Platform 1, samples need to be collected from other reefs, including the Lobos-Tuxpan reef system and Campeche Bank Reefs, and neighboring platforms.

Platform 2, the only other sampled platform to the west of the FGB, was significantly different from all other populations, except the FGB and Platform 4. It appears that both the East and West FGB are a contributing larval source to the *M. decactis* population on Platform 2. Since no genetic distinction was found between Platforms 2 and 4, it is most likely that Platform 2, located to the west, may be influencing recruitment to the east on Platform 4. With dominant circulation patterns around the FGB predominantly to the east throughout the whole year (Oey, 1995; Lugo-Fernandez, 1998; Lugo-Fernandez et al., 2001), this appears to be likely.

The *Madracis decactis* population occurring at the West FGB was homogeneous with all populations except with Platform 1. It appears that the West FGB is most likely dispersing larvae of this species to its sister bank as well as to neighboring platforms in both directions (both east and west).

Platform 3 is unique in the sense that it is located between both the East and West FGB. Indeed, it was found to be genetically homogeneous with both of the banks as well as with Platforms 5 and 7. The most probable larval source for this *Madracis* population is both the East

and West FGB due to close proximity (approximately 2 km from the EFGB perimeter; see Sammarco and Andrews, 1988; Sammarco, 1994). As for the genetic similarity shared between all five of these populations, the FGB and Platform 3 are most likely the potential larval sources for Platforms 5 and 7 located to the east. Since genetic exchange appears to be occurring between Platform 3 and both banks, this platform could be an important source for re-seeding the reefs if the population of *Madracis decactis* there experienced mortality for some reason (*e.g.*, bleaching, disease, or other anthropogenic factors). This implies that the platforms could serve as a source contributing to coral populations on the FGB, and a possible stabilizing force in the event of a disturbance.

As mentioned earlier, genetic exchange appears to be occurring in *Madracis decactis* between the two banks and the surrounding platforms. Both Platforms 2 and 3, and the West FGB lie to the west of the East FGB, indicating that there is a westward exchange of larvae. Since *Madracis* is brooder (Diekmann et al., 2001; Vermeij et al., 2003), larvae can be released monthly for 8-10 months out of the year, taking advantage of different circulation patterns in the region present at those times. By releasing larvae repeatedly over this period, the larvae have more opportunities to settle and colonize both the east and west, further expanding their range. In addition, the East FGB also appears to contribute larvae to Platforms 4 and 5 to the east.

Platform 4 may be acting as a stepping-stone (Hubbs, 1959; Kimura and Weiss, 1964; MacArthur and Wilson, 1967; Veron, 1995) between the FGB and Platform 5. It is located halfway between the populations (11 km from the East FGB and 13 km to Platform 5). The coral larvae dispersed from both banks appeared to settle on Platform 4. It is then possible for the colonies recruiting at this platform to disperse larvae further east, colonizing Platform 5.

With Platform 4 acting as a stepping-stone, larvae can be dispersed more effectively between the FGB and Platform 5.

Platform 5 appears to be a larval synch here for *Madracis*, being subjected to the dominant eastward currents, with the two FGB most likely contributing additional larvae. Platforms 3 and 4 probably act as stepping-stones in the genetic exchange of larvae to the east. In addition, Platform 5 may also be serving as a stepping-stone expanding its population to Platform 7.

Platform 6 was significantly different from all other populations except the West FGB. Similar to Platform 1, the *Madracis* population on Platform 6 could be the result of a Founder effect, with the West FGB the most likely larval contributor. Likewise, this population also had a very small sample size (n = 7), which could account for the similar pattern seen here (Ahlroth et al., 2003; Ramos-Paredes and Grijalba-Chon, 2003). It appears that the West FGB is most likely contributing *Madracis* larvae to Platform 7, with Platforms 3 and 5 acting as stepping-stones to disperse larvae to the east.

With respect to *Diploria strigosa*, the AMOVA analysis did not reveal any significant difference between the East and West FGB. Like *Madracis decactis*, the population of *Diploria strigosa* on the East and West FGB are clearly interconnected, with strong genetic cross-exchange between them. The West FGB was also found to be genetically homogeneous with Platforms 5 and 7, which again may be attributed to the dominant eastwardly current.

Data from Platform 3 suggest that the East FGB is probably dispersing *Diploria strigosa* larvae there. Either the currents in this region are transporting larvae in that direction or loop eddies are driving larval exchange. Since genetic exchange occurs between Platform 3 and both

banks, it is likely that Platform 3 serves as a stepping-stone for larval transport to Platform 7 from the FGB.

The *Diploria strigosa* population on the East FGB was homogeneous with all of the other four populations examined here, indicating that the East FGB is most likely dispersing larvae to these other populations. As mentioned above, genetic exchange is occurring in *D. strigosa* between the two FGB. Both banks appear to contribute larvae to the Platform 5 population.

Diploria strigosa is known to participate in an annual mass spawning at the FGB, broadcasting gametes into the water column, usually at the end of August or beginning of September (Bright et al., 1991; Hagman et al., 1998a, b). Unlike *Madracis decactis*, larvae dispersed by broadcast spawning have one opportunity per year to disperse their reproductive propagules. Broadcast spawning combined with the predominantly eastward current at this time of year help to explain why all of the *Diploria strigosa* colonies found on the sample platforms occurred only to the east of the West FGB; none were found to the west of the West FGB. This marks a major difference between the effectiveness of dispersal between a broadcasting *vs*. a brooding species.

The AMOVA analysis for *Montastraea cavernosa* yielded unusual results. The fact that the East and West FGB were genetically distinct from one another implies that the two banks are not genetically interconnected with regards to *M. cavernosa*. This seems counter-intuitive, since the banks have been in close proximity for thousands of years. It is improbable that no genetic exchange would be occurring in a species that has been documented to participate in annual mass spawning (Bright et al., 1991; Hagman et al., 1998a, b). One possible explanation is the East and West FGB are highly self-seeded with respect to this species, with relatively little genetic exchange occurring between the reefs.

The West FGB seems to be the most probable larval source for Platform 7 for this species. The East FGB does not appear to have contributed any of the sampled colonies to this population.

When examining the AFLPOP analysis for *Madracis decactis*, it appears that bias was introduced into the results by a limited sample size. The fact Platforms 1 and 6 had the highest percentage (>94%) of individuals allocated back to their population of origin, indicated that these two populations are highly distinct, which was also supported by the AMOVA analysis. To confirm the high levels of self-allocation rates observed in these two platforms, sample sizes of all the *M. decactis* populations were reviewed. Of all the populations examined here, these two platforms had the lowest sample sizes (Platform 1, n = 8; Platform 6, n = 7) – two to three times lower than the other sites. Although all the individuals found there were collected, sample size may simply not be large enough to reliably estimate differences between these populations and may represent another limiting factor for the AFLPOP program.

As with any statistical test, increasing the sample size will increase the power of the test; in this case, however, sample size was limited by natural abundance at these locations. The percentages of self-allocation revealed by AFLPOP analysis in the remaining populations appear to be accurate, particularly when using a minimum log-likelihood difference of one.

The AFLPOP analysis for *Diploria strigosa* yielded results similar to those for *Madracis decactis*. The three platform populations (Platforms 3, 5, and 7) had very high self-allocation; the two FGB, however, had the lowest percentages (approximately 33%). When sample size was reviewed here, all three of the platform populations were found to have extremely low sample sizes (n = 3, 4, and 5) – five to ten times lower than the FGB populations. The natural abundance of these organisms was low on these platforms, and it would appear that low sample

size at these three study sites was driving the high percentages of self-allocation. More samples would need to be collected to validate any significant differences found here.

Of the three species examined, the AFLPOP analysis for *Montastraea cavernosa* at the East and West FGB had the highest percentages (68%, 85% respectively) of colonies allocated back to its population of origin. This indicates that there is a higher rate of self-seeding occurring at the two banks and less genetic connectivity between them, supporting the AMOVA findings, indicating that the two banks were genetically distinct. Sample size did not appear to be a contributing factor in biasing the observed patterns.

In general, most coral planulae, and certainly the brooded ones, tend to settle on a reef itself or within a very short distance from it (Sammarco and Andrews, 1988, 1989; Sammarco, 1994). Coral recruitment tends to decline as distance from the natal reef increases; i.e., the probability of successful settlement is higher locally and decreases as a function of the square of distance.

Diploria strigosa and *Montastraea cavernosa* are mass spawners and participate in this activity annually (Bright et al., 1991; Gittings et al., 1992, 1994; Soong, 1993; Hagman et al., 1998a, b). Much debate has surrounded the dispersal capabilities of brooders and broadcasters. It was once thought that corals that participated in mass spawning were able to have their larvae transported over slightly longer distances by the use of currents, at least in the early phases of dispersal, allowing the larvae to take advantage of hard substrate at greater distances from the reef. Recent findings suggest that the larval dispersal of broadcast spawning corals might not be as great as was once thought (Miller and Mundy, 2003). It is now thought that the dispersal among brooding species may actually be more variable than broadcast spawners with the dispersal capability of the brooder dependent upon the larval characteristics and prevailing

currents during the planktonic phase (Harii and Kayanne, 2003). Once larvae derived from broadcasters have developed, however, the potential for long-range dispersal for larvae derived from broadcasting and brooding is most likely comparable (Sammarco, 1994, 1996; Sammarco and Andrews, 1989).

The dispersal potential of larvae is most likely determined by species-specific competency period, larval position in the water column, and currents that deliver the larvae (Harii and Kayanne, 2003). This is exemplified by the patterns yielded here by *Madracis decactis*. The larval competency of *Madracis* may be greater than that of the other two species. Most brooding corals tend to release large, well-developed planulae that contain a high lipid content (Richmond, 1981; Wilson and Harrison, 1998); while broadcast larvae develop externally are typically smaller and have lower energy reserves (Harrison and Wallace, 1990). Even though the brooded larvae are capable of settling more quickly than that of broadcast spawned larvae, the maximum competency period of brooded larvae is most likely greater due to their large energy reserves (Richmond, 1989; Wilson and Harrison, 1998). As for the size of the *Madracis decactis* planulae, it is twice as large as those found in other *Madracis* spp. (Vermeij et al., 2003); thus, it may be better suited for longer distance dispersal. *Madracis decactis* appears to have a broader geographic range than *Diploria* or *Montastraea*, possibly due to better competitive colonizing abilities. In addition, it is subject to a host of varying circulation patterns throughout the year, which seems to have a major impact on its distribution in this area.

All *Madracis decactis* colonies collected from the seven platforms occurred on platforms $\geq$ 15 years of age. Sammarco et al. (2004) found that this species is a good indicator of later stages of coral community succession. It is possible that this species may not be adapted to

compete well with species characteristic of early seres, but it is better at competing with later successional species.

As mentioned earlier, *Madracis decactis* is a brooder, releasing fully developed planulae. It has been found that some brooded offspring of marine invertebrates typically settle very close to their parents (Olson, 1985; Knowlton and Jackson, 2001). From these related results, one would not expect to find colonies of *Madracis decactis* dispersed over such a large distance range; but this process has likely been facilitated by platforms acting as stepping-stones (Hubbs, 1959; Kimura and Weiss, 1964; MacArthur and Wilson, 1967; Veron, 1995). This is best exemplified by the exceptions, strangely enough; i.e., the platform furthest west was genetically distinct from all the other populations examined, showing no homogeneity with the FGB. If this platform is not genetically interconnected with the FGB, the larvae must have been derived from some outside source over long distances, or they have are derived from neighboring platforms, which have in turn been colonized by larvae transported from an outside source. It is possible, but not likely, that this platform has been isolated by distance. Isolation occurs if the geographic range of a species is large relative to the dispersal potential of its propagules; then genetic drift will lead to the divergence between sub-populations, even at equilibrium. Another possible reason is post-settlement selection (Levinton and Lassen, 1978; Sammarco, 1980, 1982a, 1991). It is possible that all of the larvae were similar at the time of settlement and then were differentially selected out by predation such as fish grazing, competition, or some other factor. This might account for why none of the coral species sampled here were found on platforms < 15 years of age.

Coral colonies of all three species were found on Platform 7, located approximately 49 km from the nearest reef perimeter. This indicates that all of these species possess the capability to easily expand their habitat range to this distance. It also shows how other platform structures in this region have served as stepping-stones to facilitate this expansion, irrespective of mode of reproduction.

In conclusion, the coral larval stage, despite whether it is derived from a brooder or a broadcast spawner, acts as an important agent of dispersal and gene flow (DiBacco and Levin, 2000). It is believed that the brooding *Madracis* may actually have an advantage over the other two broadcasting species in terms of expanding its geographic range when suitable habitat is distributed as here at the meso-scale. The planulae of this species could be carried by an array of circulation patterns, traveling in a multitude of directions, sampled throughout the year. *Diploria* and *Montastraea*, annual mass spawners, have only one opportunity per year to disperse their larvae and can utilize only a single current pattern per year. The dominant eastern circulation in the northern GOM around the FGB probably accounts for these two species being found only to the east of the West FGB.

Corals that occur on platforms such as those examined here must be good competitors for space, because, for the most part, there is no free space on them; they are characterized by numerous species of flora and fauna (Hill-House et al., 2003). Platforms provide a mechanism by which corals can expand their populations in the Gulf of Mexico by providing hard substrate, which extends into and through the euphotic zone in an area that would otherwise be unavailable due to the depth limitations and substrate type (soft bottom; Sammarco et al., 2004). Corals utilizing platforms are only provided a limited amount of time to settle, colonize, and reproduce because the platform may be removed as result of decommissioning once the oil or gas resource

has been exhausted. Judging by the results of this study, if more artificial reefs were added to the joint federal/state "Rigs-to-Reefs" Program, the existing coral populations in the Gulf of Mexico would be expanded beyond the Flower Garden Banks even further than they are now. In addition, these platforms, acting as artificial reefs, could act as a "reserve" for re-seeding these banks if their coral populations were to become extensively depleted due to natural or anthropogenic forces.

CONCLUSIONS

The three most abundant hermatypic scleractinian corals found on seven oil and gas platforms in the northern GOM, approximately 180 km SE of Galveston, Texas, were *Madracis decactis*, *Diploria strigosa*, and *Montastraea cavernosa*. Their populations were compared to populations located on the Flower Garden Banks for degree of genetic affinity via AFLPs. The major findings of this study may be summarized as follows.

1. The Flower Garden Banks are interconnected via larval exchange for both *Madracis decactis* and *Diploria strigosa*.
2. *Montastraea cavernosa* populations at the Flower Garden Banks may be more self-contained or self-seeded due to currents or eddies in the region.
3. *Madracis decactis*, a brooder, was found on platforms to the east and west of the FGB. This suggests that by releasing planulae monthly for 8-10 months out of the year, larvae are carried by and benefit from a series of circulation patterns in the region at those times. In addition, its brooded planulae are likely to have a longer competency period than broadcast spawned larvae due to their larger energy reserves.
4. *Diploria strigosa* and *Montastraea cavernosa*, broadcast spawners, were found only on platforms east of the West FGB. This may be due to their reproductive mode and its larvae being exposed to prevailing eastward currents during the spawning season.
5. Oil and gas platforms in the vicinity of the FGB appear to be facilitating the spread of coral larvae by acting as stepping-stones, especially with regard to *Madracis decactis*.
6. Accuracy of the AFLPOP statistical analysis program appears to be limited by use of a minimum log-likelihood of zero and small sample size. A log-likelihood of one is recommended for obtaining reliable results.

For all three coral species on the East and West FGB, recruitment appears to be localized; the Banks may be self-seeded. AMOVA analyses indicated that the two banks were homogeneous with regards to *Madracis decactis* and *Diploria strigosa*. Populations of *Montastraea cavernosa* at the two banks, however, were found to be genetically distinct. AFLPOP analyses indicated that a much higher rate of self-allocation was occurring with *M. cavernosa* at the two banks, compared to the other target species. This might account for genetic distinction indicated by the AMOVA analysis. In addition, *Madracis decactis*, a brooder, appears to have a broader geographic distribution across the study sites than *Diploria strigosa* or *Montastraea cavernosa* - both broadcast spawners. This may be due to a) the monthly release of larvae over an 8-10 month period, allowing a variety of circulation patterns at those times to carry the larvae in different directions; b) longer competency periods for brooded larvae due to larger energy reserves; and/or, c) their being better competitors for space. Despite mode of reproduction, all three species possess the capability to expand their habitat range, as indicated by their colonization of Platform 7, located approximately 49 km from the nearest reef perimeter.

Future research should include the collection of adult coral samples from other neighboring reef systems, such as the Campeche Bank Reefs and the Lobos-Tuxpan reef system in the southern GOM. In addition, sampling should be expanded to other platforms in the northern Gulf. A comparison of samples from these locations with the Flower Garden Banks would be most informative, along with the determination of possible sources of larvae for the genetically distinct population of *Madracis decactis*.

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APPENDIX: EXPERIMENTAL PROTOCOLS

Protocol for Preparation of Coral Tissue Lysates

1. Add ~20 mg of coral tissue to each 1.7 ml microcentrifuge tube.
2. To each sample tube, add 275 µl of the digestion solution master mix. The components of the digestion master mix are listed below.

Digestion Solution Master Mix Components	**Volume per Sample**
Nuclei Lysis Solution	200 µl
0.5M EDTA (pH 8.0)	50 µl
Proteinase K, 20mg/ml	20 µl
RNase A Solution	5 µl
Total Volume	**275 µl**

3. Incubate the sample tubes overnight in 55ºC heat block.
4. Centrifuge samples at 2000 g to pellet undigested tissue.
5. Transfer the supernatant to a new 1.7 ml microcentrifuge tube.
6. Add 250 µl of Wizard® SV Lysis Buffer to each tube containing the supernatant and vortex to mix.

Protocol for Purification of Coral Genomic DNA from Lysate Using a Microcentrifuge

1. Transfer the entire sample lysate from the 1.7 ml microcentrifuge tube into a Wizard® SV Mini-column assembly. The mini-column assembly consists of a Wizard® SV Mini-column and a collection tube.
2. Place the mini-column assembly containing the lysate into a microcentrifuge and spin at 13,000 g for 3 minutes binding the genomic coral DNA to the Wizard® Mini-column. Spin for an additional 1 minute if lysate remains after the initial spin.
3. Remove the Wizard® SV Mini-column from the assembly and discard the liquid in the collection tube. Return the mini-column to the collection tube.

4. Add 650 µl of Wizard® SV Wash Solution to each Wizard® SV Mini-column assembly.
5. Centrifuge at 13,000 g for 1 minute.
6. Discard the liquid in the collection tube and return the Wizard® SV Mini-column to the empty collection tube.
7. Repeat steps 4-6 three more times for a total of four washes.
8. After the last wash, empty the collection tube and reassemble the Wizard® SV Mini-column assembly.
9. Centrifuge at 13,000 g for 2 minutes to dry the binding matrix.
10. Remove the Wizard® SV Mini-column and place in a new labeled 1.7 ml microcentrifuge tube for elution.
11. Add 250 µl of Nuclease-Free water to the Wizard® SV Mini-column and incubate for 2 minutes at room temperature.
12. Centrifuge the Wizard® SV Mini-column/elution tube assembly at 13,000 g for 1 minute.
13. Remove the tube assembly from the centrifuge and add another 250 µl of Nuclease-Free water to the Wizard® SV Mini-column.
14. Incubate at room temperature for 2 minutes.
15. Centrifuge the Wizard® SV Mini-column/elution tube assembly at 13,000 g for 1 minute. The total elution volume will be ~500 µl.
16. Remove the Wizard® SV Mini-column and discard.
17. Cap the elution tube containing the purified coral genomic DNA and store at -20 to -70ºC.

AFLP Protocol

Part A. Genomic DNA Digestion and Adapter Ligation

1. Make up the Restriction-Ligation "master mix" according to the number of samples to be analyzed. Before adding the adapters, heat the adapters at 95ºC for 5 minutes. Allow the adapters to cool at room temperature over a 10 minute period and centrifuge for 10 seconds. Add the components in the order shown.

Restriction-Ligation Master Mix Components	**Volume per Sample**
T_4 Ligase 10X buffer (w/ATP)	1.1 µl
0.5 M NaCl	1.1 µl
BSA (1 mg/ml)	0.5 µl
***Mse*I adapters (50 µM)	1.0 µl
***Eco*RI adapters (5 µM)	1.0 µl
*Mse*I (NEB 4 U/µl)	0.25 µl
*Eco*RI (NEB 20 U/µl)	0.25 µl
T_4 Ligase (3 U/µl)	0.33 µl
Total Volume	**5.53 µl**

2. Add the following reagents to each 1.7 ml microcentrifuge tube.

Restriction–Ligation Mix	**5.5 µl**
Genomic DNA (.5-1 µg)	**5.5 µl**
	11.0 µl

3. Mix and centrifuge for 15 seconds.

4. Incubate at room temperature overnight.

5. At the end of the Restriction-Ligation reaction, add 189 µl of TE buffer (10 mM Tris-HCl, pH 8.0 / 0.1 mM EDTA).

6. Mix well and store at -20ºC. (Tubes are template for the Preselective Amplification, Step B).

Part B. Preselective (PS) Amplification

1. Make up the preselective amplification "master mix" according to the number of samples to be analyzed. Add components in the order shown.

Preselective Amplification Master Mix Components	**Volume per Sample**
Nuclease-free water	8.1 µl
10X PCR buffer (Mg^{++} in buffer)	2.0 µl
5 mM dNTP's	0.8 µl
*Eco*RI PS primer (2.75 µM)	2.0 µl
*Mse*I PS primer (2.75 µM)	2.0 µl
Thermostable DNA polymerase (5U/µl)	0.1 µl
Total Volume	**15.0 µl**

2. To each 0.5 ml microcentrifuge tube, add 15 µl of the preselective amplification "master mix".
3. Add 1 drop of mineral oil to each tube to prevent the DNA from being evaporated during the cycling of temperatures.
4. Add 5 µl of each of the DNA samples (Digestion-Ligation from Part A) to the corresponding 0.5 ml microcentrifuge tube.
5. Mix well and centrifuge for 15 seconds.
6. Perform the preselective amplification according to the following cycle.

72ºC.....2 min……..…Initial incubation

94ºC…..20 sec….denaturation
56ºC…..30 sec….annealing
72ºC…..2 min…..extension
…20 cycles

72ºC…..2 min…….……..Final extension
60ºC…..30 min……...Final incubation

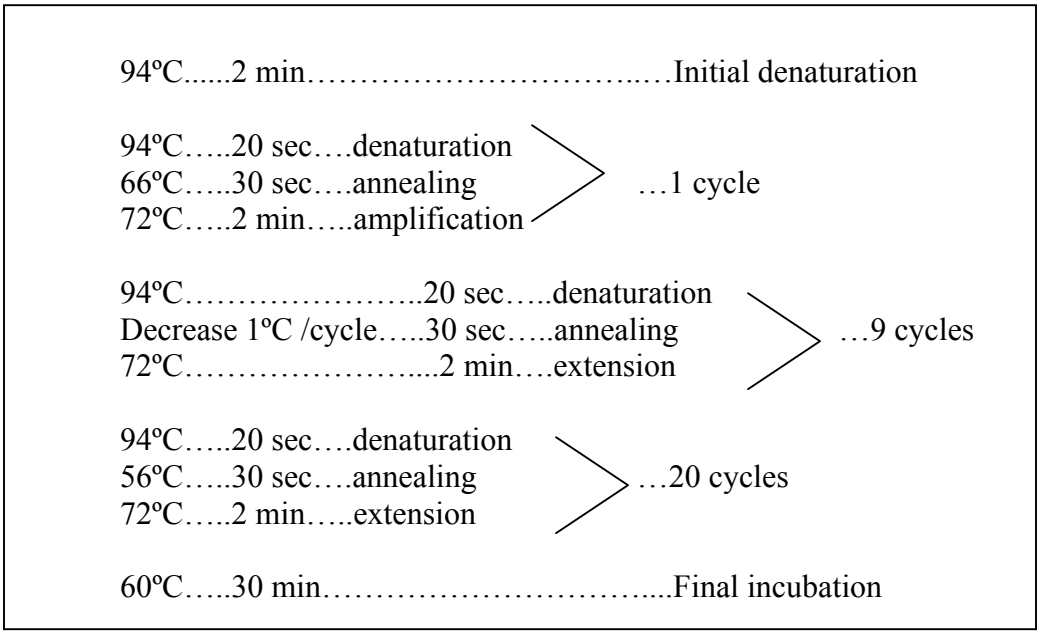
7. When PCR is completed, add 180 µl of TE buffer to each microcentrifuge tube (Tubes constitute the templates for the Selective Amplification, Step C).

Part C. Selective Amplification

1. Make up the selective amplification "master mix" according to the number of samples to be analyzed. Add components in the order shown.

Selective Amplification Master Mix Components	**Volume per Sample**
Nuclease-free water	8.1 µl
10X PCR buffer (Mg^{++} in buffer)	2.0 µl
5 mM dNTP's	0.8 µl
*Eco*RI A## primer (0.46 µM)	2.0 µl
*Mse*I C## primer (2.75 µM)	2.0 µl
Thermostable DNA polymerase (5U/µl)	0.1 µl
Total Volume	**15.0 µl**

2. To each 0.5 ml microcentrifuge tube, add 15 µl of the selective amplification "master mix".
3. Add 1 drop of mineral oil to each tube to prevent the DNA from being evaporated during the cycling of temperatures.
4. Add 5 µl of each of the DNA samples (Preselective Amplification from Part B) to the corresponding microcentrifuge tube.
5. Mix well and centrifuge for 15 seconds.
6. Perform the selective amplification according to the following cycle.



Silver Sequence™ DNA Sequencing System Protocol

Preparation of the Sequencing Plates

A. Short Glass Plate Preparation

1. Prepare fresh binding solution by adding 3 µl of Bind Silane to 1 ml of 95% ethanol, 0.5% glacial acetic acid.
2. Wipe the clean plate with Kimwipes® tissue saturated with 1 ml of freshly prepared binding solution. Cover the plate completely with the solution.
3. After 5 minutes, apply ~2 ml of 95% ethanol to the plate and wipe with a Kimwipes® tissue in one direction and then perpendicular to the first direction using gentle pressure. Repeat was three times, using a fresh towel each time, to remove excess binding solution.

B. Long Glass Plate Preparation

1. Change gloves before preparation of the long glass plate to prevent cross-contamination with the binding solution.

2. Wipe the clean plate using a tissue saturated with SigmaCote® solution.

3. After 5-10 minutes, remove excess SigmaCote® solution by wiping the plate with Kimwipes® tissue. Excess SigmaCote® may cause inhibition of staining.

C. Preparation of the Sequencing Gel

Prepare a 5% polyacrylamide gel in 7 M urea in TBE buffer.

Recipe for Polyacrylamide Gel

1. Add 52.5 ml of SequaGel Concentrate (SequaGel® Sequencing System, National Diagnostics, GA) to a 250 ml Erlenmeyer flask.
2. Add 15.0 ml of SequaGel Diluent (SequaGel® Sequencing System).
3. Add 7.5 ml of SequaGel Buffer (SequaGel® Sequencing System).
4. Add 300 µl of TEMED (*N,N,N',N'*-tetramethylethylenediamine; Gibco/BRL, Life Technologies, CA).
5. Add 600 µl of 10% APS (ammonium persulfate).
6. Mix with a stir bar.
7. Next, place the gel between 2 glass plates using a syringe.
8. Allow the gel to sit until it polymerizes.

Protocol for 10X TEB buffer

Instructions per liter:

1. Add 108 g Tris base.
2. Add 55 g Boric acid.
3. Add 9.3 g EDTA
4. Add 1000 ml of water.
5. Stir on a plate for ~30 minutes.

D. Silver Staining the Sequencing Gel

Preparation of the solutions.

1. Prepare the following solutions.
   a) Fix/stop solution (10% glacial acetic acid): Add 200 ml of glacial acetic acid into 1,800 ml double-distilled water. Do not reuse fix/stop solution.
   b) Staining solution: Combine 2 g (1 packet) of Silver Nitrate ($AgNO_3$) and 3 ml (1 vial) of 37% Formaldehyde in 2 L of double-distilled water.
   c) Developing solution: Dissolve 60 g (1 packet) of Sodium Carbonate ($NaCO_3$) in 2 L of double distilled water. Chill to 10°C in an ice bath. Immediately before use (Step 6a), add 3 ml (1 vial) of 37% Formaldehyde and a 400 µl aliquot of the provided Sodium Thiosulfate (10 mg/ml). Discard remaining Sodium Thiosulfate in the vial.
2. After electrophoresis, carefully separate the plates using a plastic wedge. Gel should be attached to the short glass plate.
3. Place the gel (plate) in a shallow plastic tray, cover with fix/stop solution and agitate well for 20 minutes or until the tracking dyes are no longer visible.
4. Rinse the gel 3 times (2 minutes each) with double-distilled water using agitation. Lift the gel (plate) out of the wash and allow it to drain 10-20 seconds before transferring to the next wash.
5. Transfer the gel to staining solution and agitate well for 30 minutes.
6. a) Complete preparation of the developing solution by adding 3 ml (1 vial) of the provided 37% Formaldehyde and 400µl aliquot of Sodium Thiosulfate (10 mg/ml) to the pre-chilled (10°C) Sodium Carbonate solution. Pour 1 L (half) of the pre-chilled developing solution into a tray and set it aside. Keep the remaining developing solution on ice.
   b) Remove the gel from the staining solution and set it aside. Transfer the staining solution

into a flask or beaker for silver recovery. Rinse the tray and fill it with double-distilled water.

7. Dip the gel briefly into the tray containing the double-distilled water, drain and place the gel immediately into the tray of chilled developing solution. Time taken to dip the gel in the water and transfer to developing solution should be no longer than 5-10 seconds.
8. Agitate the gel well by rocking until the template band starts to develop or until the first bands are visible. Transfer the gel to the remaining 1 L of chilled developing solution and continue developing for an additional 2-3 minutes or until all bands become visible.
9. To terminate the developing reaction and fix the gel, add 1 L of fix/stop solution (from Step 3) directly to the developing solution and incubate with shaking for 2-3 minutes. Longer incubations will fade the stain.
10. Rinse for 2 minutes each time in double-distilled water.
11. Leave the gel at room temperature. View the gel on a light box at visible wavelengths.

VITA

Amy Atchison was born in West Monroe, Louisiana, on September 19, 1979. She graduated from Cedar Creek High School in Ruston, Louisiana, in 1997. She attended Louisiana Tech University and received a Bachelor of Science in biology and a minor in chemistry in February 2001. In the summer of 2001, she enrolled in graduate school in Louisiana State University's Department of Oceanography and Coastal Sciences. She is now a candidate for a Master of Science degree in the spring of 2005.