Investigation of connectivity among coral communities using population genetic analysis
E.F. Cox, R.A Kinzie III, T.D. Lewis, and S.P. Kolinski, Hawai‘i Institute of Marine Biology
Grant No. 6-52618
31 January 2005

Executive Summary

Understanding the extent of dispersal of marine larvae is critical to evaluate the assumption that Marine Protected Areas can serve a vital function for replenishing depleted populations in adjacent or more distant regions. One approach to understanding connectivity of populations is the use of population genetics to describe subpopulation structure and to estimate migration rates between populations. The goal of this project was to investigate population genetics of a keystone species within Kāne‘ohe Bay, O‘ahu, Hawai‘i, as a first step to understanding larger patterns of connectivity at the scale of the entire Hawaiian Archipelago. We focused on the scleractinian coral species, Pocillopora damicornis. This species is a common reef flat species, with brooded planulae. Although the assumption has been that brooded planulae should be associated with local dispersal, these larvae are capable of extended pelagic existence. In addition, early protein allozyme studies suggested that the planulae are clonally derived from the brood parent. Asexual reproduction complicates the analysis of population structure.

We used microsatellites to attempt to describe population structure in P. damicornis. Microsatellites are short repeated segments that have become powerful tools for identifying population genetic structure. Additionally, these markers can identify clonal relationships between individuals. The microsatellites we used were identified from studies of the conspecific Pocillopora meandrina. However, we found that these microsatellites do not have significant variability in populations of P. damicornis and therefore were unacceptable for analysis of population structure in this species. Although we were unsuccessful in describing population structure in this species, the issue is still clearly an important management question. We have begun to search for nuclear intron markers that can serve as acceptable population level markers for P. damicornis. The unresolved question of the extent to which asexually produced larvae determine the pattern of population structure in this species of coral must be answered, even though this species, the “lab rat” for much research on coral physiology, is difficult to address with the current methodologies available.

Purpose

A pressing question for the field of conservation ecology involves the dispersal of marine organisms (Warner and Cowen 2002). This is especially critical for the body of theory and experiments that is currently being used to justify the creation of Marine Protected Areas (MPAs). As most marine organisms have a pelagic stage, the paradigm
had been to assume extensive dispersal occurs and that local populations receive their
recruits from a homogeneous larval pool, even if dispersal was sporadic in time and space
(Jackson 1986). Species with brooded larvae or demersal eggs and larvae were the
exceptions to this paradigm, primarily because of the limited dispersal potential of their
larvae. However, recent evidence suggests that local retention of larvae is more common
than expected, even in species that have a long pelagic larval stage (Swearer et al. 2002,
Hellberg et al. 2002). This question has major consequences for conservation ecology.
For example, MPAs are expected to serve as sources of new recruits to sustain
populations in other areas. The extent of dispersal of larvae is vital information to
evaluate this assumption. One goal of HCRI is to “improve understanding of population
structure and recruitment of keystone reef organisms” in order to address links between
life history patterns and genetic population structure.

Scleractinian corals are keystone reef organisms. They provide the foundation for
coral reefs. They are one of the major reef builders, provide shelter and food for other
reef organisms, and are components of the primary productivity of reef food chains.
Understanding the connectivity and genetic structure of coral populations will be critical
to predicting the trajectory of reef recovery.

Scleractinian corals demonstrate both sexual and asexual reproduction. Asexual
reproduction frequently occurs by fragmentation or fission of adult colonies (Highsmith
1982) but also can result from asexual production of larvae (Stoddart 1983, Resing and
Ayre 1986, Stoddart et al. 1988) or polyp “bail-out” (Sammarco 1982). Asexual
reproduction should result in low levels of genotypic diversity within local populations
(Lasker and Coffroth 1999) and can also decrease effective population size (Hughes et al.
1992). In corals, sexual reproduction involves spawning of male and female gametes for
external fertilization and development or brooding of larvae within the polyp of the
parent and subsequent planulation. The assumption has been that planulation of large
brooded larvae decreases long-range dispersal of genotypes, as larvae will tend to settle
close to the parent (Jackson 1986). In contrast, external spawning has been assumed to
result in planulae with a greater ability to disperse from the parent. Therefore population
structure should reflect this difference in larval type, with high genetic relatedness within
species with brooded larvae compared to species with externally spawned gametes (Ayre
and Hughes 2000).

However, recent work (Ayre and Hughes 2000) with both spawning and brooding
corals along the Great Barrier Reef has shown that the simple prediction of reduced
dispersal in brooding species is not always supported. For the nine species they
surveyed, local populations of both brooding and spawning species appeared to maintain
local population structure through self-seeding, although gene flow was sufficient to
prevent fixation of genetic differences. These results are similar to studies of other
scleractinian species, including Pavona cactus (Willis and Ayre 1985, Ayre and Willis
1988), Acropora spp. (Ayre et al. 1991), Porites compressa (Hunter 1993), Seriatopora
hystrix (Ayre and Dufty 1994), Balanophyllia elegans and Parcycanthus sternsii (Hellberg
1996), Pocillopora verrucosa (Ridgway et al. 2001), Plesiastrea versipora (Rodriguez-
Lanetty and Hoegh-Guldberg 2002), and Fungia fungites (Gilmour 2002). These studies
have found evidence of local population structure coupled with some degree of
connectivity between populations.
Likewise, studies of other marine populations have shown that self-recruitment into local populations is more common than previously supposed (Swearer et al. 2002). Studies typically find that levels of retention, and hence genetic similarity, are high on local and ecological time scales, but sufficient gene flow occurs to maintain panmixia in populations on broad and evolutionary time scales.

An increasingly important method of assessing population structure is the use of microsatellite markers. These markers are short, randomly repeated motifs (1-6 base pairs) found throughout the genome. Given the high variability in number of repeats, these markers can serve to distinguish closely related individuals (Sunnucks 2000), and therefore these markers can be used to identify clone members in corals. Although initially difficult to find in corals (Marquez et al. 2003), they have recently been identified for several species of corals (Maier et al. 2001, Le Goff & Rogers 2002, Miller & Howard 2004, Magalon et al. 2004, Severance et al. 2004).

**Goals and Objectives**

The goals of this study will be
1. to assess local patterns in population structure a common scleractinian species in Kāne‘ohe Bay,
2. to quantify the relative importance of sexual and asexual reproduction within populations, and
3. to compare recent recruits to these populations with planktonic larvae as well as adult populations within the local area.

**Site and Species Selection**

Hawai‘i, and Kāne‘ohe Bay in particular, can serve as a model system in which to test the connectivity between populations and the contribution of sexual and asexual reproduction to population dynamics. The Hawaiian Archipelago is one of the most isolated in the Pacific Ocean and has a high degree of endemism in its marine fauna. The Hawaiian scleractinian fauna includes species with broad geographical ranges, such as *P. damicornis*, as well as endemic species, including *P. compressa*. In the latest revision of the checklist for shallow-water scleractinians (Maragos 1995), 14 of 50 species of Hawaiian corals are listed as probably endemic.

Kāne‘ohe Bay, the location of the Hawai‘i Institute of Marine Biology (HIMB), has been characterized as “one of the most intensively studied coral reef systems in the world” (Hunter and Evans 1995). Its physical oceanography, primary productivity, coral reef ecology, and interactions with the surrounding watershed have been well studied (for example, Smith et al. 1981, Hunter and Evans 1995, Laws and Allen 1996, CISNet 2002). Prior to 1978, domestic sewage was discharged into the southern basin of Kāne‘ohe Bay, and changes in the nutrient loading have been a major focus of experimental research in the Bay (Smith et al. 1981). Kāne‘ohe Bay has also periodically been impacted by storms that increase stream discharge by orders of magnitude (Banner 1968, Jokiel et al. 1993). Additional impacts currently under study include the impact of pulsed stream flow on nutrient dynamics and the distribution and ecology of alien species.
Kāne‘ohe Bay is semi-enclosed and approximately 4 km wide and 13 km long. It is partially protected from oceanic swell by a “barrier reef”. There are two major channels into the Bay, the northern deep ship channel (approximately 15m depth) and a shallower southern channel (approximately 5-10m depth). Approximately 50% of the area consists of the lagoon floor, covered with fine sediments. The remainder consists of fringing and patch reefs and the barrier reef. The Bay has been subdivided into three major zones, differing in their connectivity to the ocean and degree of water motion. The southern basin, previously directly impacted by sewage discharge, has limited direct connection to the ocean and lower flushing times. The central basin lies directly behind the barrier reef, and is flushed daily by wave-driven flow over the barrier reef and tidal currents through the southern channel and out the northern ship channel. The northern basin receives strong waves across the barrier reef, as the depth of this structure in the northern basin is 7 to 10 m, and is rapidly flushed through tidal cycles.

*Pocillopora damicornis* is abundant within Kāne‘ohe Bay. *P. damicornis* releases large brooded planulae throughout the year (Jokiel 1985, Jokiel et al. 1985, Kolinski and Cox 2003). Larvae typically settle rapidly following planulation but can survive for extended periods. During the 1980s, the reef flat at HIMB was dominated by two color morphs, Y and B, that released planulae at different periods during the lunar cycle (Richmond and Jokiel 1984).

*Pocillopora damicornis*, the “lab rat” for many coral studies, is a geographically widespread species. Early studies of *P. damicornis* in Kāne‘ohe Bay and Rottnest Island (Western Australia) suggested that this species has a high degree of clonality, with clones consisting as many as 115 members (Stoddart 1984a&b, 1986, 1988). Using protein electrophoresis, Stoddart (1983, 1986) also found that planulae had identical phenotypes to the parent. Martin Chavez (1983) prepared histological sections of *P. damicornis* and found both oocytes and spermataries within polyps but only 2 spermataries in all of his sections were mature, while all others appeared to be degenerating before maturity was reached. These two types of evidence have been used to assert that planulae are primarily asexually produced in populations of *P. damicornis* in Hawai‘i.

In contrast, Benzie et al. (1995), Ayre et al. (1997) and Ayre and Hughes (2000) found that the multi-locus genotypic diversity, described by protein electrophoresis, of *P. damicornis* populations along the length of the Great Barrier Reef could only be explained by sexual reproduction. They also described inbreeding of these populations, presumably related to the assumed low dispersal capabilities of coral sperm and/or planulae. Ayre et al. (1997) suggested that the importance of asexual reproduction in this species may vary geographically, with a predominance of asexual reproduction at the range margins. In Japan, Adjeroud and Tsuchiya (1999) found evidence of asexual reproduction in populations of *P. damicornis*, probably associated with fragmentation in wave-swept reef flat habitats. Although asexual reproduction was important for local population maintenance, they demonstrated gene flow between their sampling sites.

There is also some controversy about the potential for larval dispersal in this species. Harrigan (1972), Richmond (1987) and Harii et al. (2002) have maintained brooded larvae in laboratory situations for as long as 212 days. Although most larvae are believed to settle within hours or days of release (Harrigan 1972), Hodgson (1985) found *P. damicornis* larvae frequently in plankton tows within Kāne‘ohe Bay. These observations have been used to argue that *P. damicornis* has a high potential for long-range dispersal.
Approach

Field Collections:

Adult colonies were haphazardly collected from the reef flat adjacent to the Point Laboratory at the Hawai‘i Institute of Marine Biology for planulae collection. Colonies were placed in small holding containers with flowing seawater. Seawater exiting the containers was passed through a planulae collector, a 250ml plaster beaker with plankton mesh panels. Planulae were retrieved from the collectors daily and used for DNA extraction. Branch tips from the adults were also sampled for DNA.

A 5 x 5 m field plot at the north end of Coconut Island (study site #6 in Stoddart [1983]) was established, and locations of all P. damicornis colonies mapped within the grid (Figure 1). Two branch tips from all colonies were sampled; one was processed for DNA and one was frozen in liquid nitrogen.

Figure 1: location of all 68 P. damicornis colonies within a 5 x 5m study plot (same as Site #6 in Stoddart [1986]) at Coconut Island.

DNA Extraction:

High molecular weight genomic DNA was isolated from samples using DNeasy™ Tissue kits from Qiagen (Valencia CA). The manufacturer’s procedure was followed with the following modifications for adult coral samples. Coral pieces were incubated in the extraction buffer at 55°C overnight, until the outermost cell layer was released into the solution, and at no time were the samples vortexed as shearing of the DNA may occur. The final elution of gDNA from Qiagen mini-columns was performed in two steps. The first elution with 100 µl buffer was collected sequentially and kept separate from a second 50 µl volume elution. Integrity of the gDNA was assessed electrophoretically on a 1% agarose gel and visualized with SYBR Green (Molecular Probes, Eugene OR) using a UV-transilluminator. These samples are quantified by H33258 incorporation using a spectrophotometer.
Microsatellites

PCR amplifications of pocilloporid microsatellites (Magalon et al. 2004, Table 1) were performed on a BioRad™ thermocycler using 2 µL DNA template, 4 µL primers (5µM), 4 µL H2O and 10 µL BioOne BioMix™, with 1.5 µM MgCl₂, for a total volume of 20 µL. A touchdown cycling protocol was used (Table 2). PCR products were fluorescently labeled with ABI dyes and run on an ABI sequencer with a 400 bp size standard. Allele size was estimated using the ABI fragment analysis software.

<table>
<thead>
<tr>
<th>Table 1: Pocilloporid microsatellite primer information. Number of alleles based on Magalon et al. (2004).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Locus</strong></td>
</tr>
<tr>
<td>PV2</td>
</tr>
<tr>
<td>PV5</td>
</tr>
<tr>
<td>PV6</td>
</tr>
<tr>
<td>PV7</td>
</tr>
<tr>
<td>Table 2: PCR protocol</td>
</tr>
<tr>
<td><strong>Step</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
</tbody>
</table>
Findings

Microsatellites

Microsatellites were successfully amplified from DNA of 3 adult colonies and 10 planulae from each. There was no variability between adults and their own larvae, however, there was also no variability among alleles in the adults sampled. Likewise, in the large set of 68 samples from Coconut Island, there was no variability in alleles at 2 of the 4 loci (Figures 2 & 3), 1 locus was difficult to amplify (Figure 4), and the 4th locus showed only 2 alleles (Figure 5). Ten samples from northern Kane‘ohe Bay and 12 samples from Olowalu, Maui (provided by Dr. Frank Stanton) also showed no additional alleles for these markers.

Figure 2: position of alleles within the 5 x 5 m study plot at Coconut Island for microsatellite marker Pv2 – blue squares: individuals with 160bp product; red triangles: individuals with no scorable product.

Figure 3: position of alleles within the 5 x 5 m study plot at Coconut Island for microsatellite marker Pv6 – blue squares: 205bp; red triangles: no scorable product.
These microsatellite markers were developed for a different species of pocilloporid, and therefore the lack of variability when used in *Pocillopora damicornis* is not totally unexpected, as microsatellites occasionally will amplify in related species but without the variability in number of alleles found in the species for which they were developed.

We still have samples from these 68 individuals in the freezer that could be analyzed using protein electrophoresis, a method used extensively with this species (Miller and Ayre, 2004, Ayre and Miller in press), because of the difficulty in finding acceptable molecular markers for population studies in this species (Miller, pers. comm.).
We are also initiating studies to identify suitable nuclear introns that have sufficient variability to serve as within population markers for *P. damicornis*.

**Evaluation**

Unfortunately we were unable to meet our goals for this project. Because we were unable to find sufficient variability in the microsatellite markers we were applying to this species, we were unable to quantify the contribution of asexual and sexual reproduction to population structure in this species. Two new papers, again using protein electrophoresis, have suggested that although larvae of *P. damicornis* in Australia (Miller and Ayre, 2004; Ayre and Miller in press) are asexually derived from the parent, the overall adult population recruits primarily via sexual reproduction. This poses an important paradox for this species: why do individuals expend energy in the substantial production of asexual larvae when the majority of recruitment into the population occurs via sexual reproduction and how are sexual recruits produced in this species? The unresolved question of the extent to which asexually produced larvae determine the pattern of population structure in this species of coral must be answered, even though this species, the “lab rat” for much research on coral physiology, is difficult to address with the current methodologies available.

Assessing levels of exchange between populations is a critical management issue, especially with the growing threats to coral keystone species associated with global climate change. Predicting the re-establishment of populations following bleaching events, for example, will become a major factor in the next decade. We are confident that additional molecular markers will become available to answer these important questions in Hawaiian scleractinian species.

**Literature Cited:**


Signature

Evelyn F. Cox
Hawai‘i Institute of Marine Biology