PRELIMINARY RESULTS OF AN INVESTIGATION OF GENETIC STRUCTURE WITHIN HAWAIIAN MICRO-MOLLUSK COMMUNITIES

Jeff Eble

Introduction

Within the past decade, Hawai`i and much of the world has seen a significant increase in attempts to mitigate pressures on various important or endangered marine species and habitats through increased regulation and the establishment of Marine Protected Areas (MPA). Current marine conservation efforts within the Hawaiian archipelago include the proposal (under review) to designate all federally controlled waters of the Northwest Hawaiian Islands as a National Marine Sanctuary. While within state waters a total of ten Marine Life Conservation Districts (MLCD) have been established throughout the islands and another nine Fisheries Replenishment Areas (FRA) have been designated along the West Hawai`i coastline (pamph. Fishing Reg., Dept. Aquat. Reg.). The establishment of MPAs is typically a politically charged issue, in part because of the speculative nature of resource management. The ecological relationships of species to their respective environments are complex and generally not well understood. Ultimately, the success of current and future marine resources management strategies is dependant on the accuracy of the assumptions we make regarding the dynamics of marine populations.

Most marine species have life histories that include at least one potentially widely dispersing stage. For benthic species, dispersion is typically accomplished via the passive drift of pelagic larvae. Numerous studies have utilized advances in molecular biology to examine how larval dispersal affects the population structure of marine species along linear coast lines (Hedgecock 1986, Williams and Benzie 1993, Edmonds et al 1996, Marko 1998, Wares et al 2001) and throughout the Pacific (Palumbi 1996, Williams and Benzie 1993), though few have examined how larval dispersal affects population structure of species within a complex archipelago [exception, Zink et al1996 (endemic Hawaiian gobies) and Johnson et al 2001 (Siphonaria kurracheensis)].

The current paradigm for gene flow between populations of benthic organisms holds that the temporal and spatial scale of ocean currents connects populations via long-distance dispersal of larval stages. The realization of high dispersal potential is supported by the presence of larval offspring from various near shore species in an average of 92% of the deep ocean plankton tows conducted (Palumbi et al 1997). Palumbi (1996), in a study of pacific wide genetic structure of the tropical urchin, genus Echinometra, discovered indistinguishable mtDNA sequences in individuals separated by as much as 4800 km. Similar patterns of genetic homogeneity were observed by Williams and Benzie (1993) in a study of the long-distant disperser Linckia laevigata, and by Kay and Palumbi (1987) in an analysis of 28 cytosolic allozyme loci of the Giant Clam (Tridacna maxima), which were found to exhibit 97% homogeneity in populations separated by up to 1000 km.

Though there exists a number of studies supporting the contention that larvae are transported regularly between populations, the complex dynamics of the marine environment limits the usefulness of generalizations. The dispersal potential of the various larval developmental strategies of marine invertebrates represent a continuum ranging from mere centimeters for the solitary coral Balanophylla elegans (Hellberg 1984) to transoceanic crossings [genus Echinometra (Palumbi 1996)]. Hellberg's (1996) study of two closely related solitary corals (Balonaphylla elegans and Paracyathus sternsii) with different dispersal patterns (non-planktonic brooder and free spawner respectively), but otherwise similar life histories highlights the contribution of reproductive strategy to population genetic structure. Hellberg observed strong subdivision both within and between the populations of Balonaphylla with 31% of the genetic variation within the Balonaphylla population accounted for by distance, while distance was found to be responsible for only 1% of the variation within the Paracyathus population. The differences in population heterozygosity suggests an apparent connection between the length of planktonic larval phase and degree of population genetic structuring, where any increase in dispersal capabilities results in an associated decrease in population heterogeneity.

Genetic exchange between populations is ultimately the product of both species and environmental dispersal potential. Each environment has a distinct effect on dispersal within and between resident populations, discriminating against or supporting particular behavioral or re-

productive strategies. The degree of dispersal, whether controlled by the individual or the environment, will affect species geographic range (Wares et al 2001), rates of colonization (Palumbi 1996), and levels of gene flow (Hedgecock 1986). A thorough characterization of how differences in reproductive strategy and habitat influence the population dynamics of marine species is essential to the establishment of sound management strategies.

In this study, mitochondrial DNA (mtDNA) sequence data was used to reconstruct haplotype phylogenies of Hawaiian populations of the direct developing neogastropod Peristernia chlorostoma. The current assumption is that Hawai`i's marine population exhibit panmixia, with disparate populations exchanging genes regularly [reef fish (Hourigan and Reese 1987), corals (Jokiel 1987), and non-scleractinian invertebrates (Kay and Palumbi 1987)], yet the majority of studies to date were limited in their ability to resolve the degree of gene flow between populations due to a dependence on traditional systematic techniques (i.e. Comparisons of morphological and life-history differences). As a direct developer, P. chlorostoma is limited in long distance dispersal to random anthropogenic or natural rafting. The constraints placed in P. chlorostoma dispersal provides us with the most sensitive test of Hawaiian marine population structure, thus establishing a maximum genetic differentiation from which we might later base a comparison of the effects different reproductive strategies have on the degree of gene flow between Hawaiian marine populations.

Methods

Collection of Samples

Between June and September 2002, samples were collected from six locations spanning a distance of greater than 2000 km. Coordinates for sampled localities, in descending order of longitude, are as follows: Pearl and Hermes Atoll, NWHI (27°50'N, 175°50'W); Salt Pond Park, Kauai (21°54'N, 159°36'W); Olowalu, Maui (20°50'N, 156°63'W); Hawai`i – Hilo (HIL) (19°45'N, 155°30'W); Kapoho Coral Gardens (KCG) (19°30'N, 155°00'W), Mahukona (MHK) (20°11'N, 155°54'W). Distance between adjacent localities ranged from 25km (HIL and KCG) to 1500km (Kaua`i and MWHI) with no greater than 500km between main island sampling sites. At each locality, samples were collected from depths not greater than 2m below mean low water. Samples were fixed and preserved in 70% ETOH until processing.

Electrophoresis

Genomic DNA was extracted from foot tissue dissections using animal tissue extract protocol for Qiagen's Dneasy tissue kitÐ Sequences were obtained for a 600bp segment from the cytochrome c oxidase subunit 1 (CO1) mitochondrial gene using primers CCO1490 (GTTCAACAAATC ATAAAAGATATTGG) and CCO2198 (TAAACTTCAGGGTGAC-CAAAAAATCA). Amplification was performed in a 25-µl total reaction volume with 1.5µl of each primer (10.3µM), 2.5µl dNTPs (2.0mM), .2µl BSA, 2.5µl 10x buffer, 2.8µl $MgCl_2$ (25mM), .25µl Taq polymerase, and 1.5µl DNA. Samples were heated to 95°C for 3 minutes followed by 35 cycles (denaturation of 95°C for 30s, annealing at 42°C for 30s, and extension at 72°C for 1 minute) and a final extension at 72°C for 3 min, in a PTC-1000 Programmable Thermal Controller DNA amplifier. PCR products were purified by electrophoresis in .9% agarose gels. A significant number of the samples showed non-target amplification (both 1000bp and 500bp sequences were observed). Target sequences were isolated from agarose with Qiagen's QIA quick PCR purification kit.
Sequencing was conducted with the Thermo Sequenase Cycle Sequencing Kit (USB) in a 18µl total reaction volume with 1.25µl flourescently labeled CCO1490 primer (10.3µM), 1.5µl dNTPs (2.0mM), .5µl BSA, 2µl sequenase buffer, 2µl thermo-sequenase, and 4µl DNA. Automated DNA sequencing was conducted in the LI-COR Gene Reader 4200 (NEN).

Analysis

Alignment and proofing of sequence data was conducted using AlignR version 1.2. Determination of within and among population nucleotide diversity and haplotype analysis was carried out in PAUP version 4.0b10.

Results

Sequence Variation

A fragment of 600bp from the CO1 mtDNA gene was obtained from 24 individual Peristernia chlorostoma. Sixteen different haplotypes were identified. 67 variable sites were found (11%), 40 of which were fixed in individual populations (Fig. 2). Of the total mutations recorded, only 1 yielded a non-synonymous change.

Transitions accounted for 96.6% of the substitutions; the adenine: thymine ratio was high (62%). Degree of with population sequence divergence correlates with island geological history, with populations from older islands exhibiting greater within population genetic distance. (Fig. 1).

Phylogenetic Analysis

The comparison between the different likelihood scores for each model of evolution showed that the HKY model (Hasegawa et al 1985) was the best-fit model among those evaluated for our data. This model incorporates unequal base frequencies [(A)=0.2420, (T)=0.3902, (C)=0.1701, (G)=0.1977], and a transition/transversion ratio (s/v = 9.8309). Haplotypes are shared within populations but not between populations. A neighbor-joining haplotype tree revealed 6 clades (Fig. 3), representing each of the 6 sampled populations.

Limited sampling of the North West Hawai`i Islands restricts our comparison of within population divergence to main island samples. Maximum population pair wise distance recorded was 6.9% [samples 94 (NWHI) and 95 (Maui)]. Pair wise distance between populations within the main islands (Kaua`i, Maui, and Hawai`i) was only slightly lower at 6.7% [samples 98 (Kaua`i) and 11 (Maui)]. Genetic distance between clades increased with geographic distance; the most diverged clades being the NWHI and Hawai`i Island HIL and KCG samples. Pair wise distance between adjacent sites HIL and KCG on the island of Hawai`i was recorded to be .01901, or just greater than the average within population genetic distance recorded (.01778), yet significantly smaller than genetic distance observed between samples within populations from Kaua`i and Maui (.03477 and .02577 respectively).

Discussion

Population Subdivision

At all spatial scales examined, Peristernia chlorostoma showed high levels of genetic subdivision, inconsistent with the assumption of limited genetic structure within Hawaiian marine invertebrate populations (Kay and Palumbi 1987, Jokiel 1987). The dispersive potential of marine environments has long been thought to limit genetic subdivision over all but the greatest of distances. This was supported by the few genetic studies of Hawaiian marine populations conducted to date (Winans 1980, Shaklee and Samollow 1983, Shaklee 1984). The existence of significant genetic differentiation between Hawai`i island populations, HIL and KCG (separated by only 10km along a linear coast line), suggests, at least for non-pelagic dispersers, there exists significant constraints

to gene flow between even adjacent populations. Our observation of genetic differentiation over limited geographic range is not unique. A number of studies have revealed local genetic subdivision in response to physical barriers to gene flow (Marko 1998), site-specific selection (Rowan et al 1977), and species dispersal capabilities (Hellberg 1996, Collin 2001).

The presumption of high gene flow between Hawaiian invertebrate populations was based principally on an examination of morphological and reproductive differences. The systematics of marine gastropods is notoriously convoluted, with species and subspecies regularly being split and joined. The historical chaos of gastropod systematics is evidence of the limited utility traditional systematic techniques can play in an investigation of the degree of genetic subdivision within a given population. Traditional examinations of morphological and behavioral differences often lack the resolution necessary to identify the degree of gene flow between all but the most diverged populations. The lack of distinct morphological or behavioral differences between populations is not in itself evidence of homogenizing gene flow. Even at geological time scales, divergence of populations is not always represented by coinciding differences in observable characteristics. Genetic composition, nature of selective pressures, and the inherent randomness of mutations all combine to drive the evolution of a population. Sometimes this produces overt signs of underlying genetic divergence, though more often the change remain hidden to all but the most resolved analysis.

Phylogenetic Analysis

The colonization and subsequent divergence of subpopulations from source populations establishes patterns of genetic similarity and differences that, when compared, can be highly useful in the determination of phylogeographic relatedness. The genetic composition of any given population is the sum of three primary factors: 1, the genetic composition of founding individuals; 2, degree of gene flow between disparate populations; and 3, site specific effects on allele frequencies. Each factor leaves tell tale marks on an individual's DNA that can be examines in infer a population's life history.

With P. chlorostoma populations there appears to exist a correlation between clasal genetic and geographic distance; the greater the geographic distance the more divergent the populations. Genetic distance between clades represents a lineage map with the most divergent being the most distantly related, the least divergent being the most closely related, and so on. The apparent connection between geographic and genetic distance within Hawaiian populations of P. chlorostoma suggests a linear progression of colonization, with initial colonization at one end of the island chain followed by subsequent island hopping colonization events. Alone, the data can only reveal the extent of relatedness and not actual phylogeny.

The origin of present day P. chlorostoma populations remains speculative, yet a comparison of within population genetic divergence suggests progressive colonization from the north. The older the population, the more time is has had to accumulate mutations, thus establishing a reference frame from which we can estimate relative times of colonization. Of the main island populations, maximum pair wise distance between individuals within a population appears to correlate with population longitude; maximum in Kaua`i and minimum in KAP (Hawai`i), supporting the theory of colonization from the north via the Kuroshio extension of the North Pacific Equatorial Current. Introduction from the north has been proposed for a variety of Hawaiian marine taxa, including corals (Jokiel 1987) and near-shore fish, which show strong affinity with the Ryuku Islands and southern Japan (Hourigan and Reese 1987).

Isolation of the Hawaiian archipelago within the middle of the North Pacific Gyre limits possible colonization routes. Besides the Kuroshio extension, the only major current system affecting the islands is the Sub-tropical Counter-Current, which extends from west to east, encountering the Johnson Stoll at its terminus. The patterns of within population genetic distance we've recorded in Hawaiian P. chlorostoma suggest initial introduction from the south was unlikely. Populations sampled from the island of Hawai`i exhibited the lowest within population genetic distance, contrary to what would be exhibited if P. chlorostoma had initially colonized Hawai`i and then spread northward. Affinity of the Johnston Atoll coral community to the Hawaiian islands rather than with its nearest up-current neighbor, the Marshall Islands (Jokiel 1987) suggests the north to south colonization route may represent a common dispersal path for Hawaiian invertebrates.

Further resolution of observed patterns required a more thorough sampling of individuals and sites. Assuming that the transport of individuals between populations via rafting of with marine traffic is negligible, as a non-planktonic disperser, connectivity between populations of P. chlorostoma is limited to crawling distance, a highly restrictive mechanism for genetic exchange. This considered, we would expect there exists genetic differentiation at a scale of <10km (as observed between HIL and KCG throughout P. chlorostoma's range.

Whether the degree of genetic structure we've observed in our initial project is supported by further investigations not only will impact our interpretations of the population structure and life history of Hawaiian Peristernia, but as well may have a significant impact on management strategies for a number of important Hawaiian marine species. As the pressures on Hawai`i's marine environment increase, there exists an ever-greater need to resolve the phylogeographic structure of Hawai`i's marine populations. Management strategies that do not take into account the life history and population structure of target species may yield limited benefits or may, inadvertently, inhibit conservation efforts.

The results of our initial research have shown the assumption of high gene flow between populations of Hawaiian invertebrates to be false, highlighting the need to further characterize how behavioral, reproductive, and geographic differences between species and populations may effect Hawaiian marine community dynamics. Only through the implementation of management strategies based on sound research might we ultimately protect Hawai`i's unique communities.

Fig. 1 - Map of Hawaii showing approximate location of the different sampling sites. Number in parentheses denotes within population nucleotide differentiation.

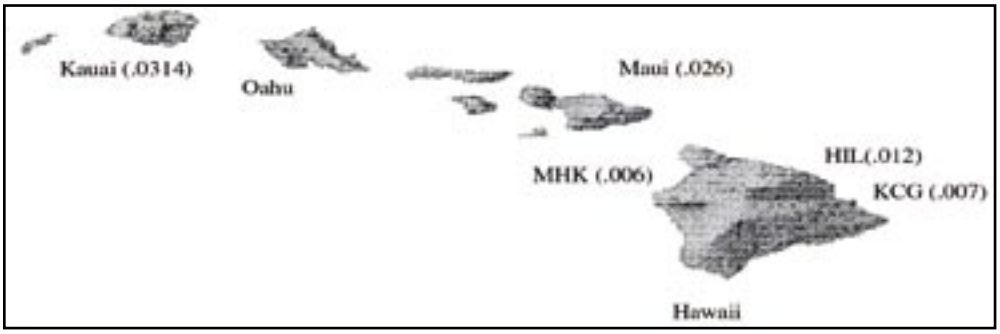
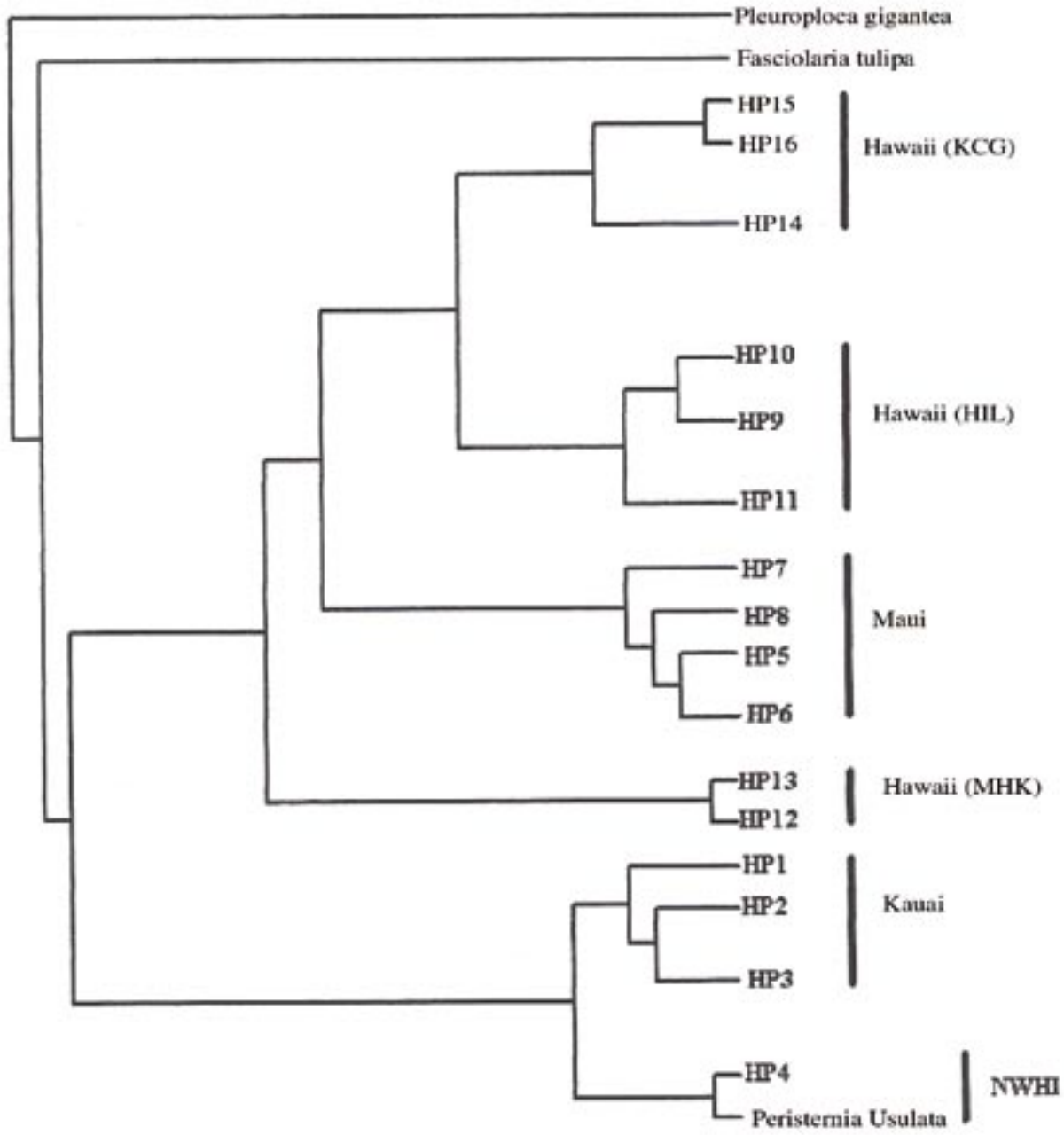


Fig. 2 - Sequence variation among 16 mtDNA haplotypes for 600bp of the CO1 gene in Peristemia chlorostoma. Only those positions differing from the consensus are shown. A number at the top (read vertically downward) indicates codon changed. Number of individuals belonging to each of the haplogypes is shown in parentheses next to haplotype name. A dot indicates a base pair identical to that of the consensus sequence, and a letter represents a change in the nucleotide. First position changes are shown in bold, all other changes were in the third codon position. Non-synonomous changes are identified with an asterics.

			1	1	1	1	1	2	2	2	2	3	3	4	4	5	5	5	5	5	6	6	6	7	7	7	7	8	8	8	8	9	9	9
		T	1	2	3	4	6	0	2	4	5	0	2	6	7	2	3	4	6	9	1	4	5	3	4	T	3	1	5	6	8	1	2	3
Hep I				C				A	.	T		T		T		C		A	G	C	A		C	C		C	.	G			T			
Hep II				C				A	.	A		T						.	.	C	.		C	C		C	.	.			T			T
Hep III	(3)			C				A	.	A		T						.	.	C	.		C	C		C	.	.			T			T
Hep IV				C		A		A	C	.	C	.						.	.	C	.	T				C	C	G		G			A,T	
Hep V													A	T											C								A	
Hep VI	(2)												A	T											C									
Hep VII					T								.	T											C								A	
Hep VIII													A	T											C								A	
Hep IX			C				A	C	A																C				G				A	
Hep X			C				A	C	.																C				G				A	
Hep XI	(2)	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Hep XII		T	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Hep XIII	(2)	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Hep XIV	(4)	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Hep XV		T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Hep XVI		T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	G	.	.
Concensus		C	T	T	C	G	T	G	T	C	A	C	G	C	C	T	G	G	A	T	G	C	T	T	T	T	T	A	C	A	C	A	G	C
				1	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
		3	**3**	**9**	**9**	**8**	**9**	**8**	**1**	**1**	**2**	**2**	**2**	**3**	**3**	**3**	**4**	**4**	**5**	**5**	**5**	**5**	**5**	**5**	**6**	**6**	**6**	**6**	**6**	**8**	**8**	**8**	**3**	**3**
		7	**3**	**0**	**1**	**2**	**4**	**7**	**0**	**7**	**9**	**8**	**3**	**1**	**5**	**7**	**1**	**3**	**8**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**5**	**6**	**1**	**4**	**5**	**6**	**7**
Hep I		.	A	.	A	G	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	C	.	C	C	.	.	G	C	
Hep II		A	A	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.	.	
Hep III	(3)	A	A	.	.	.	.	T	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	C	G	C	
Hep IV		.	.	.	A	.	C	.	.	.	.	.	C	T	C	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	C	G	C	
Hep V		.	A	.	A	.	.	.	.	C	.	.	.	.	.	C	.	.	C	.	.	.	.	G	G	.	A	.	C					
Hep VI	(2)	.	A	.	A	.	.	.	.	C	.	.	.	.	.	C	.	.	C	.	.	.	.	G	.	.	A	.	C					
Hep VII			A	.	A	.	.	.	.	.	.	C	.	.	.	C	.	.	C	.	.	G	.	.	.	.	.	.	.					
Hep VIII			A	.	A	.	.	.	.	C	.	.	.	.	.	C	.	.	C	.	.	.	.	G	.	.	A	.	C					
Hep IX				C					C											.	G												C
Hep X		.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	C
Hep XI	(2)	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.
Hep XII		.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
Hep XIII	(2)	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.
Hep XIV	(4)	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.
Hep XV		.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	A	.	.	.	.
Hep XVI		.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G
Concensus		G	G	G	G	A	T	C	T	T	A	T	T	C	T	T	T	T	T	A	A	A	A	A	A	T	G	T	G	T	T	A	T	T

Fig. 3 Neighbor joining tree for the haplogypes, estimated using the model of evolution HKY. Hawaii Island populations are Mahukona (MHK), Hilo (HIL), and Kapoho Coral Gardens (KCG).



Acknowledgements

This student research was conducted under the direction of Dr. Marta DeMaintenon, University of Hawai`i at Hilo Assistant Professor in Marine Science, as part of UH-Hilo's Research Experience for Undergraduates in Tropical Conservation Biology, a program funded by the Nation Science Foundation, aimed at increasing research opportunities for undergraduates.

Literature Cited

Edmunds, D., Moberg, P.E. and Burton, R.S. 1996. Allozyme and mtDNA evidence of population subdivision in the purple sea urchin Strongylocentrotus purpuratus. Marine Biology 126: 443-450

Hasegawa M., Kishino K., Yano T. 1985. Dating the human-ape splitting by a molecular clock of mitochondria DNA. Journal of Molecular Evolution 22: 160-174

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science 39: 550-564

Hellberg, M.E. 1984. Relationships between inferred levels of gene flow and geographic distance in philopatric coral, balanophyllia elegans. Evolution 48: 1829-1854

Hellberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution 50: 1167-1175

Hourigan, F.H., Reese, E.S. 1987. Mid-ocean Isolation and the Evolution of Hawaiian Reef Fishes. Trends in Ecology and Evolution 2: 197-191

Johnson, M.S., Bentley, S.L., Ford, S.S., Ladyman, M.T., & Lambert, G.J. 2001 Effects of a complex archipelago on genetic subdivision on the intertidal limpet imphonaris kurracheensis. Marine Biology 139: 1987-1094

Jokiel, P.L. 1987. Ecology, Biogeography and Evolution of Coral in Hawaii. Trends in Ecology and Evolution 2(7): 179-182

Kay, E.A. & Palumbi, S.R. 1987. Endemism and evolution in Hawaiian marine invertebrates. Trends in Ecology and Evolution 2(7): 183-186

Marko, P. 1998. Historical allopatry and the biogeography of speciation in the prosogranch snail genus Nucella. Evolution 52: 757-774

Palumbi, S.R. 1996. What can molecular genetics contribute to marine biogeography? An urchin's tale. Journal of Experimental Marine Biology and Ecology 203: 75-92

Palumbi, S.R., Grabowski, G., Duda, T., Geyer, L. & Tachino, N. 1997. Speciation and population genetic structure in tropical Pacific sea urchins. Evolution 51: 1506-1517

Rowan, R., Knowlton, N., Baker, A.J., Jara. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388: 265-269

Shaklee, J.B. 1984. Genetic variation and population structure in the Damselfish, Stegastes fasciolatus, throughout the Hawaiian Archipelago. Copeia 3: 629-640

Shaklee, J.B., Samollow, P.B. 1983. Genetic aspects of population structure of four species in the Northwestern Hawaiian Islands. Sea Grant Technical Report: 264-276

Wares, J.P., Gaines, S.D. & Cunningham, C.W. 2001. A comparative study of asymmetric migration events across a marine biogeographic boundary. Evolution 55: 295-306

Williams, S.T. & Benzie, J.A.H. 1993. Genetic consequences of long larval life in the starfish Linckia laevigata (Echinodermata: Asteroidea) on the Great Barrier Reef. Evolution 55: 295-306

Zink, R.M., Fitzsimmons, J.M., Dittmann, D.L., Reynolds, D.R. & Nishimoto, R.T. 1996. Evolutionary genetics of Hawaiian freshwater fish. Copeia 1996: 330-335