MOLECULAR APPROACHES TO MARINE ECOLOGY AND EVOLUTION

Organizers: Howard R. Lasker, Mary Alice Coffroth and Eldredge Bermingham

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Molecular Approaches to Marine Ecology and Evolution

Introduction

B9-001 CONSISTENCY, IDIOSYNCRASY, AND THE CONCEPT OF PHYLOGEOGRAPHIC DEPTH IN CONSERVATION BIOLOGY, John C. Avise, Department of Genetics, University of Georgia, Athens, GA 30602.

The genetic structure of geographic populations can range from evolutionarily shallow to deep. Deep structures are likely to characterize species whose populations have experienced limited or no genetic contact over longer (evolutionary) timescales; these define the major intraspecific units of potential conservation relevance. The empirical hallmark of deep structure is a consistency of population partitions as registered by multiple, independent genetic characters. Such genealogical concordance can have four aspects, involving: (a) multiple characters within a gene; (b) multiple genes within a species pedigree; (c) multiple co-distributed species that have been impacted by similar historical biogeographic events; and (d) agreement with zoogeographic provinces as defined by species' distributional limits. Shallow population structures are likely to characterize any species in which single-generation dispersal distances of individuals are much less than the geographic range occupied by the species, and these too can be relevant to conservation interests. The empirical hallmark of shallow structure is an idiosyncrasy of population partitions across multiple, independent genetic markers. An argument will be developed that the matrilineal component of population structure (as registered for example by mitochondrial DNA) should be of special significance to conservation biology because of an unusually tight genetics-demography connection.

B9-002 NEW FRONTIERS IN MOLECULAR MARINE BIOLOGY: OPPORTUNITIES FOR THE TWENTY FIRST CENTURY, Dennis A. Powers, The Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950

The oceans cover more than 70% of the earth's surface and considering the great depths of the ocean, this aquatic habitat contains over 95% of the world's biosphere by volume. The tremendous biodiversity in this aquatic environment is essentially an infinite reservoir of model organisms for fundamental biological research and a tremendous resource for commercially important products. Even though the application of molecular technologies toward addressing problems in aquatic environments has been modest when compared to those efforts devoted to terrestrial systems, there have already been some notable successes and preliminary evidence that the entire field of molecular marine biology is becoming a new frontier of explosive scientific discovery with exciting global scientific and economic implications for the twenty first century. Use of molecular technologies will not only provide fundamental information about the basic biology of marine organisms but they will revolutionize our understanding of the evolutionary mechanisms that regulate populations, species, and community structures. The coupling of molecular techniques with automated analyses can dramatically advance our ability to obtain fundamental knowledge about marine organisms and oceanographic processes, which are otherwise unobtainable by conventional means. Exploitation of these molecular capabilities will provide a new understanding of: (i) basic biological structures and processes, (ii) the causes of biological variability in the ocean, (iii) the implications of this variability to regional and global processes and the role of environment and climate change, (iv) the molecular mechanisms responsible for evolutionary change, and (vi) the linkage between molecular mechanisms, cellular processes, organismic, population, species, and community structure level processes. Since the dearth of fundamental information on marine organisms is the major impediment for future advances in marine biology and biological oceanography, I will emphasize how molecular approaches have yielded major advances in marine biology and point to promising research areas where molecular approaches have the potential for major contributions in the future. Some of the most promising molecular techniques and their potential for automation will be discussed in the context of specific examples where they have been particularly useful or where there is a tremendous potential for addressing fundamental evolutionary problems in relation to problems in marine biology, marine ecology, biological oceanography, conservation biology, fisheries biology, and the molecular mechanisms that regulate development, growth, and reproduction.

The Ecology and Evolution of Fertilization I

B9-003 PATTERNS OF SPAWNING AND FERTILIZATION AMONG MARINE INVERTEBRATES, John Pearse, Institute of Marine Sciences, University of California, Santa Cruz, CA 95064

Patterns of spawning and fertilization are extremely diverse among marine invertebrates. Often viewed as most typical is broadcast spawning of both eggs and sperms, followed by fertilization in the water column, and recent work has focused on how species-specific fertilization might be maximized. However, most species either broadcast spawn sperms while retaining eggs, or transfer sperms (or eggs) between parents; fertilization takes place in, on, or near the parent. Particular patterns appear to be fixed in some taxa (e.g., turbellarians, peracaridian crustaceans); events are extremely variable in others, even among closely related species (e.g., spionid polychaetes, asterinid asteroids). Correlation with trophic level, habitat, or biogeography is weak, while the influence of adult body size, growth form, and developmental mode can be strong, but only in some groups. Such a diversity of patterns presents a rich challenge, amenable to molecular approaches, for unraveling complexities in life history evolution, with major implications for understanding population structure, speciation and extinction, and gene flow both within and between species.

B9-004 BIOLOGY, BIOCHEMISTRY, STRUCTURE AND MOLECULAR EVOLUTION OF A SPERM PROTEIN MEDIATING FERTILIZATION IN ABALONES, V.D. Vacquier[1] and C.D. Stout[2], [1] Marine Biology Research Division, Scripps Institution of Oceanography, UCSD, La Jolla, CA 92093-0202 and [2] Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037.

Molecular recognition between proteins which mediate fertilization in free spawning marine invertebrates is known to restrict cross-species fertilization. The molecular evolution of fertilization proteins could therefore be involved in the speciation process. Abalone sperm lysin is a 16kDa acrosomal protein that creates a hole in the egg vitelline layer (VE) by a nonenzymatic process. The sperm swims through the hole to fuse with the egg. The dissolution of the egg VE by lysin is species-selective, not species-exclusive. This selectivity can be quantitatively assayed using purified lysin and isolated VEs. The crystallographic structure of lysin from the red abalone, *Haliotis rufescens* is known to 1.9 angstroms resolution. Lysin is composed of five alpha-helices in a tight, unique bundle configuration with loops connecting the helices (there is no beta-sheet). There are three striking features of the structure. First, the amino-terminal segment of residues 1-12 extends away from the helical bundle. Second, two tracks of Arg and Lys run down one side of the protein, giving the structure a large positive electrostatic potential. Third, a patch of 11 solvent-exposed hydrophobic residues lies on the opposite surface from the basic tracks. The amino acid sequence of abalone sperm lysin of 27 species (of the 60-70 extant species) was deduced from the cDNA sequences. Residues 1-12 are always hypervariable and species-specific. Most inter-species variation occurs in the interhelical loops and on the external faces of helices. Lysin phylogeny divides into two major clades, one composed of California and Japanese species, and one composed of species from the rest of the world. Analysis of nucleotide substitution in pairwise comparisons of lysins shows that the evolution of lysin is promoted by strong positive Darwinian selection. The selective force responsible for such a robust demonstration of positive selection remains unknown. Most other examples (about 12 are known) of positive selection involve molecular recognition phenomena in pathogenesis and immunity. The VE protects the egg and embryo from microbes. The VE may change in evolutionary time in response to microbial pressure. The sperm which interact most successfully with an ever-changing VE may be positively selected for. This idea explains how the species-selectivity of fertilization can be maintained as species divergence continues.

The Ecology and Evolution of Fertilization II

B9-005 RAPID EVOLUTION OF BARRIERS TO FERTILIZATION IN INSECTS: PARALLELS TO MARINE SPECIES, Daniel J. Howard, Department of Biology, New Mexico State University, Las Cruces, NM88003.

In marine animals that do not interact behaviorally prior to gamete release, gamete recognition loci appear to evolve quite rapidly and differences at such loci are often critical in isolating closely related taxa. The potential for rapid evolution of gamete interactions or interactions between sperm and the female reproductive tract exists in terrestrial organisms, such as insects, that undergo internal fertilization, but such signalling systems have received little attention from evolutionists interested in the evolution of reproductive isolation. In part, this lack of attention can be attributed to a lesson that evolutionists learn early in their training; namely, that many taxa that do not hybridize in nature will mate and produce fertile hybrids in the laboratory. Traditionally, this result is attributed to the breakdown of behavioral differences under artificial conditions and is taken to indicate that post-insemination barriers to fertilization rarely exist between closely related taxa. Unfortunately, this perception fails to take into account the nature of barriers to fertilization.

After insemination, successful fertilization depends on a complex sequence of interactions among male gametes, the female reproductive tract, and female gametes. Current evidence suggests that there is some species-specificity in all of these interactions, although the barriers often are not absolute and can be overcome by high concentrations of sperm. In general, the concentration dependence of barriers to fertilization has been overlooked by evolutionists designing laboratory hybridization experiments. Several recent studies of insects in which numbers of matings were controlled and sperm utilization patterns were monitored have begun to change this situation. The results indicate that interactions between sperm and the female reproductive tract or sperm and eggs can evolve quickly and that post-insemination barriers to fertilization may reproductively isolate closely related insect species from a wide variety of taxa. In my talk, I will describe these new data from insects, concentrating particularly on work in my own laboratory on the closely related ground crickets, Allonemobius fasciatus and A. socius.

B9-006 FERTILIZATION SUCCESS AMONG BROADCAST SPAWNING BENTHIC INVERTEBRATES: THE INTERACTION BETWEEN CLONAL PROPAGATION, FLOW REGIME AND SUCCESS, Howard R. Lasker and Mary Alice Coffroth, State University of New York at Buffalo, NY 14260.

Fertilization rates among broadcast spawning species are dependent on local sperm density around eggs, which in turn is dependent on a complex mix of position, current speed and direction and sperm release rate. The net effect of these interactions are fertilization rates that can vary from 0.0 to near 100%. Minimizing this variance and realizing predictably non-zero fertilization rates may be an important selection pressure on many species. For many sessile species position relative to current regime is probably unpredictable over time or may be unpredictable for settling larvae. This unpredictability can be reduced by vegetative propagation. Vegetative spread of a clone has the effect of bet hedging because the genet can spawn at several different locations simultaneously. Such an effect is apparent in fertilization rates of the Caribbean gorgonian *Plexaura kuna*, which at some sites produces clones consisting of hundreds of individuals. In the absence of vegetative propagation *P. kuna* would be rare. Fertilization rates among *P. kuna* and *Pseudoplexaura porosa* have been determined during synchronous spawning events at two sites in the San Blas Islands, Panama over the past three years. At both sites the *P. kuna* population is composed of a large widely distributed female clone, one large male clone, and at least one more distant and less numerous male clone. Fertilization rates of *P. kuna* eggs ranged from 0.0 to over 80% depending on the direction and speed of the current. The distribution of female and male clones over a range of locations increases the likelihood that some gametes are released in the immediate vicinity of those of the opposite sex. We are using RAPD analyses to determine the parentage of *P. kuna* planulae, and test the hypothesis that fertilization is determined by clone size and distribution and thus that clonal propagation can enhance fertilization rates. The effects of vegetative spread are less important among common species. *P. porosa* colonies are not clonal, but are distributed in higher local densities and commonly have higher fertilization rates than *P. kuna*.

B9-007 ECOLOGICAL AND GAMETE-LEVEL FACTORS INFLUENCE *IN SITU* FERTILIZATION SUCCESS IN SEA URCHINS, Don R. Levitan, Florida State University, Tallahassee.

In order to understand how gamete- and molecular-level factors influence fertilization success of free-spawning organisms, we need to have a basic understanding of the factors that mediate fertilization in the sea. Over the past ten years evidence has accumulated indicating that the physical environment and number of spawning animals greatly influence local sperm concentration and thus the frequency with which eggs are fertilized. Given how these two factors affect sperm concentration, and given the relative importance of sperm concentration in dictating the likelihood of fertilization, how important are the more subtle aspects of gamete quality in influencing the chance of fertilization? I used a series of experiments to correlate the performance of gametes in the laboratory and field and observed that much of the variation in intraspecific fertilization success in the field could be attributed to how well an individual's gametes performed in the laboratory. These results demonstrate that egg traits such as target size and receptiveness to sperm, and sperm traits such as swimming ability and longevity, are important factors in determining *in situ* levels of fertilization. These results also suggest that molecular gamete recognition systems, which can often be overwhelmed by high sperm concentration in the laboratory, may not be swamped by environmental factors in the field.

B9-008 DETERMINANTS OF COMPETITIVE MALE FERTILIZATION SUCCESS IN BENTHIC MARINE INVERTEBRATES, Michael A. McCartney, Section of Evolution and Ecology, University of California, Davis, CA 95616. In most marine organisms, the processes of spawning and fertilization have not been studied in natural populations. As a result, we have only a vague understanding of mating dynamics and how they influence individual reproductive success, levels of gene flow, spatial patterns of kinship, and selection on alternative reproductive strategies. The emerging field of marine fertilization ecology has concentrated mostly on elucidating hydrodynamic and other factors in the marine environment that may impact the efficiency with which free-spawned eggs are fertilized. To date, little attention has been paid to the measurement of *individual* variation in male or female fertilization success. We do not know whether fertility variation is due entirely to external factors, or rather whether it is predictably associated with variable reproductive traits and strategies, and hence may have evolutionary consequences. Since species in most marine invertebrate phyla release sperm or both eggs and sperm into the water column, determination of individual fertilization success in natural populations is difficult, but can be approached using neutral molecular markers in parentage assignment . My talk will describe how I have used genetic markers for paternity determination in manipulative field experiments with a benthic marine invertebrate. This approach has allowed me to examine two previously-unstudied issues: (1) the fertilization success consequences of sex allocation in an hermaphroditic animal , and (2) the operation of sperm competition in free-spawning invertebrates.

My work has focused on the assessment of individual *male* fertility variation, since this is most poorly understood. Some marine invertebrates invest a sizable fraction of their biomass into the formation of male gonad, but it is unknown whether investment varies or whether increased investment enhances fertilization success. I used the cheilostome bryozoan *Celleporella hyalina* in my studies. This species, a colonial hermaphrodite, confines gonads within separate male and female zooids which are morphologically distinguishable, so that male versus female gonadal investment (sex allocation) as well as somatic investment can be estimated non-invasively in living colonies. I arranged colonies with known levels of sex allocation on artificial arrays placed near natural populations, and used unique allozymes to assign paternity in brooded embryos harvested from these colonies. I present evidence that male fertilization success increases substantially with investment in male gonad. I also show that multiple male-functioning colonies compete intensively for fertilizations under natural conditions. Finally, I describe how I used my results to evaluate sex allocation theory for the maintenance of hermaphroditism in this bryozoan.

My results show that male fertility variation is reliably associated with gonadal investment, and suggest that other reproductive traits may influence male or female fertilization success in marine invertebrates. Marine species display a tremendous variety of modes of reproduction and spawning strategies, and we understand very little about their adaptive significance. For example, the timing and synchrony of spawning, various gamete characters, modes of mating and sperm transfer, and fertilization mechanisms themselves await further studies of why they have evolved in particular cases. Molecular techniques could be of great utility in studies of these and other novel issues in marine reproductive ecology and evolution.

Development

B9-009 CELLULAR INTERACTIONS DURING SEA URCHIN DEVELOPMENT, R. Andrew Cameron, Andrew Ransick and Eric H. Davidson, Division of Biology, California Institute of Technology, Pasadena, CA 91125

That the sea urchin embryo possesses an exquisite capacity to regulate development after disturbance emphasizes the central role which cellular interactions play in the patterns of spatially restricted gene expression. Early invariantly positioned cell divisions segregate unique groups of founder cells which become the primordia for the exclusive territories of gene expression. Recent studies which combine cell lineage observations, blastomere manipulations and gene expression analysis are beginning to demonstrate how cellular interactions lead to the specification of regionally restricted gene expression.

B9-010 HOW GENES INTERPRET DEVELOPMENTAL INFORMATION IN THE SEA URCHIN EMBRYO, Eric H. Davidson, Division of Biology, California Institute of Technology, Pasadena, CA 91125

This presentation concerns the mechanisms by which spatial and temporal information is decoded by *cis*-regulatory systems operating early in sea urchin embryogenesis. The relatively simple embryonic processes characteristic of indirectly developing echinoid embryos, and the excellent technologies that have been developed for gene transfer, *viz.* whole mount *in situ* hybridization, and isolation, purification and cloning of embryo transcription factors, have made this an experimental system of primary importance for analysis of gene regulation in early development. We have characterized the regulatory systems controlling several genes that are activated early in embryogenesis. These are *SM50,* which encodes a skeletogenic matrix protein gene; *CyIIIa,* which encodes a cytoskeletal actin expressed only in embryonic and larval aboral ectoderm; and *Endo 16,* which encodes a surface protein of the embryonic gut. Recent work reveals a modular functional and structural organization of the *cis*-regulatory systems driving the regulated expression of these genes.

B9-011 HOMEOBOXES IN INVERTEBRATES, Matthew H. Dick, Department of Biology, Yale University, New Haven, CT 06511.

The mechanistic bases of ontogeny are beginning to be understood at the molecular genetic level, and a revolutionary result has been the realization that metazoans with different body plans and modes of development employ structurally and functionally conserved classes, and even systems, of developmental regulatory genes. A challenge to evolutionary biologists is to understand how modification-with-descent of developmental regulatory systems maps onto patterns of phyletic change of organisms. This has a direct bearing on marine ecology. Most metazoan phyla are entirely or predominantly restricted to the marine environment, and many of them include ontogenies involving continuous development, i.e., organisms with alternation of asexual and sexual generations and/or clonal propagation by iteration of zooids, often with polymorphism. Understanding the evolutionary forces which shape these ontogenies, and their response to environment to produce temporal and ecophenotypic variation, would clearly benefit from knowledge of developmental mechanisms.

Homeobox genes are among a number of classes of genes encoding transcription factors involved in developmental pattern formation. HOM/Hox type homeobox genes are involved in specification of axial positional information during development across disparate animal phyla. They are organized in clusters; gene order in clusters has been conserved through evolutionary time; and this order of genes is colinear with the anterior boundaries of regions over which they exert developmental control. In some cases, mutations in HOM/Hox genes produce phenotypes which mimic phylogenetic change. Variation at four levels, that of homeodomain sequence, cluster composition, cluster number, and expression pattern, provide a fertile field from which to correlate phenotypic change with changes in developmental regulation.

HOM/Hox genes have been physically mapped in a few unitary organisms previously established as genetic model systems. Our approach has been to use a PCR-based strategy to survey the complement of HOM/Hox genes in representatives of such disparate organisms as cnidarians, horseshoe crab, annelids, and bryozoans; to use this information to infer cluster organization to the extent possible without mapping; to generate the reagents necessary for examining expression patterns of selected genes; and to use these reagents for comparative studies of pattern formation. Here I summarize the results of previous and ongoing studies and discuss their implications.

Larval Dispersal

B9-012 IDENTIFYING THE PLANKTONIC PLAYERS, Thomas D. Kocher, Dorothy Medeiros-Bergen, Janet A. Conroy, Nicole T. Perna, and Richard Randolph Olson, Department of Zoology and Center for Marine Biology, University of New Hampshire, Durham, NH 03824.

Identification of the larval forms of many taxa has been a problem in field studies for many years. There is a need for a species-level identification protocol which is simple and can process large numbers of individuals in a short period of time. We have developed methods to identify echinoderm larvae using the sequence of their mitochondrial 16S rRNA gene. A standard set of oligonucleotide primers can be used to amplify a hypervariable segment of this gene from larvae at any stage of development. The sequence of this fragment can be compared to a database of sequences derived from adult specimens. Intraspecific variation is low, allowing the development of species-specific oligonucleotide probes for high capacity dot-blot assays. In recent work, we have extended this approach to a hypervariable segment of the nuclear 28S rRNA gene. The D3 expansion segment is proving useful for the identification of more divergent taxa, such as acoels and nematodes. We envision a new set of tools for the identification of poorly studied, less charismatic organisms. A standardized set of primers could be used to amplify and sequence a particular region from the animal to be identified. This sequence could be electronically compared to a database of previously sequenced specimens. Matches to this database could provide identification, or at the least connect researchers working on closely related species.

B9-013 MOLECULAR CUES FROM THE ENVIRONMENT CONTROLLING SITE-SPECIFIC RECRUITMENT: MORPHOGEN-BASED "FLYPAPER" FOR LARVAE AS A TOOL FOR MANIPULATIVE EXPERIMENTS, Daniel E. Morse, Aileen N.C. Morse and Peter T. Raimondi, Marine Biotechnology Center, Marine Science Institute, University of California, Santa Barbara, CA 93106.

Planktonic larvae of benthic species from three phyla (the scleractinian coral, *Agaricia humilis*; the gastropod mollusc, *Haliotis rufescens*; and the polychaete annelid, *Phragmatopoma californica*) recognize signal molecules from the environment that induce substratum-specific settlement and metamorphosis. These signal molecules exhibit significant homology or cross-talk with the ligands of mammalian receptor systems. Recent experiments show that recognition of these chemical cues plays a major role in determining the distribution of recruits in the natural environment; the resulting patterns of recruitment are either "associative" or "gregarious," depending on the source of the signal recognized. In *Haliotis* larvae, associative settlement and metamorphosis are controlled by two convergent chemosensory pathways that respond to two different chemical signals from the environment; one is a peptide associated with the surfaces of the recruiting crustose coralline algae; the other is an amino acid dissolved in the seawater. The interaction of these two pathways, similar to the mechanism of long-term potentiation in mammalian brain, provides a capacity for fine-tuning larval selection of settlement sites. To resolve the relative contributions of chemosensory recognition and other environmental factors that control recruitment of *Agaricia humilis*, we have developed a morphogen-based chemical "flypaper" containing the purified inducer bound to an artificial substratum. The inducer is a sulfated glycosaminoglycan associated with cell walls of the recruiting crustose red algae; the chromatographically purified morphogen alone is sufficient to induce larval attachment and metamorphosis. Both the source of the inducer and responsiveness to its presence are specific: (1) the inducer is obtained only from algae that are inductive when intact; and (2) it induces metamorphosis only in species that are induced to settle by the intact algae. The amphipathic morphogen retains activity when tightly bound to beads of a hydrophobic-interaction chromatography resin, and this activity is identical in the ocean and the laboratory. Attachment of the resin-bound inducer to surfaces yields a potent artificial recruiting substratum corresponding to a morphogen-based chemical flypaper for *A. humilis* larvae. This material is proving useful in experiments resolving the role of chemosensory recognition in site-specific settlement, metamorphosis, and recruitment. It also should provide a useful tool to investigate the possibility that changes in the specificity of morphogen recognition by the cognate larval receptors serve as an axis for niche-diversification and speciation.

Population Structure I

B9-014 POPULATION STRUCTURE OF A CLONAL CARIBBEAN GORGONIAN, Mary Alice Coffroth and Howard Lasker, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York.

Clonal reproduction is a common life history strategy among sessile marine invertebrates. The clonal structure of such populations can provide insight into the ecological and evolutionary history of the population, but requires markers that identify individual genets. Using DNA fingerprinting we examined the population structure of a clonal gorgonian soft coral, *Plexaura kuna*, in the San Blas Islands Panama and in the Florida Keys. We identified 69 genotypes on 8 reefs in the San Blas Islands. All populations were multiclonal, but no genotypes were shared between reefs. Clone size (and hence population number) varied an order of magnitude between reefs with similar numbers of clones. This variation in clone size is a function of different physical environments both within and between reefs. The presence of similar numbers of genotypes on each reef suggest a steady, albeit low, level of sexual recruitment with population growth being a function of vegetative spread of some clones and not a function of recruitment. A different pattern was observed in the Florida Keys where 23 genotypes were identified. In comparison to Panama, there were few colonies and clone size was small. Population growth here is most likely a function of higher recruitment and/or post-settlement survival and not vegetative spread. Data on the size and distribution of clones allow us to make inferences about the ecologic and evolutionary aspects of the population dynamics and show that techniques such as DNA fingerprinting are essential in identifying population structure of species like *Plexaura kuna*. The data reveal not only the ubiquity of clonal growth but also that the importance of a clonal growth strategy is only realized in certain environments.

B9-015 POPULATION STRUCTURE AND THE EVOLUTION OF SOCIAL BEHAVIORS IN BENTHIC INVERTEBRATES, Rick Grosberg and Don Levitan, Center for Population Biology, University of California, Davis, CA 95616 and Department of Biological Science, Florida State University, Tallahassee.

Depending on a combination of physical processes, as well as reproductive biology, populations of sessile marine invertebrates can exhibit genetic structure on a variety of spatial scales, ranging from thousands of kilometers to millimeters. In turn, the spatial scale at which genetic structure exists influences the likelihood of genetic (and adaptive) differentiation, the frequency and intensity of intraspecific competition, the likelihood that kin will interact, and the potential for the evolution of a spectrum of social behaviors. In this talk, we first document the relationship between reproductive patterns and genetic structure in four species of benthic colonial marine invertebrates, and then consider the implications of these relationships for the evolution of social behavior. Allozyme and PCR-RAPD markers show that populations of the hydrozoan *Hydractinia milleri* and the compound ascidian *Botryllus schlosseri* are highly genetically structured. A congener of *H. milleri*, *Hydractinia symbiolongicarpus* (which lives on hermit crab shells), features kin structure, but only at very fine spatial scales. The fourth species (the anthozoan *Anthopleura elegantissima*) displays little, if any, kin structure. As a result of dense larval settlement and/or clonal growth, all four of these species compete intraspecifically for space. The nature and outcomes of these competitive interactions vary according to the relatedness of conspecifics, along with phylogenetic constraints on behavioral repertoires. In general, closely related individuals either fuse, or behave passively toward one another, whereas more distantly related individuals either actively reject one other or behave aggressively. In the two species with relatively high levels of kin structure (*i.e.*, *H. milleri* and *B. schlosseri*) intraspecific interactions lead to intergenotypic fusion or rejection (but not aggression). Although closely related *H. symbiolongicarpus* colonies often fuse to form stable genetic chimeras, more distantly related individuals either form unstable chimeras, or behave aggressively. Finally, different clones of *A. elegantissima*, at least upon first contact, behave aggressively, and never fuse. With such a limited sample of taxa, it is not yet possible to determine whether there is, in fact, any significant relationship between genetic structure and social behavior, much less whether such a relationship reflects phylogenetic history or the action of selection. Nevertheless, the preliminary comparison suggests that in order to understand how aggression and cooperation evolve in clonal invertebrates, it will be necessary to combine microevolutionary analysis of genetic structure with phylogenetic analysis of character evolution.

B9-016 POPULATION GENETIC CONSEQUENCES OF LARGE VARIANCE IN REPRODUCTIVE SUCCESS FOR MARINE ANIMALS, Dennis Hedgecock, University of California, Davis, Bodega Marine Laboratory, Bodega Bay, CA 94923-0247.

The great fecundity and high mortality in early life stages of most marine animals makes possible substantial variance in the number of offspring contributed by individual parents to the subsequent breeding population (V_k). For these species, an assumption of binomial or Poisson variance in offspring number may be wrong; instead, a small minority of individuals may replace an entire population in each generation by a sweepstakes-chance matching of reproductive activity with oceanographic conditions conducive to spawning, fertilization, larval survival, and successful recruitment. The likelihood of a mismatch between reproduction and permissive oceanographic conditions may well mean reproductive failure for most individuals. The resulting population variance in reproductive success may thus be orders of magnitude larger than binomial or Poisson variance. The primary genetic consequence of large variance in reproductive success is a decrease in the effective size of the population (N_e) relative to the breeding number (N), according to the relationship $N_e = (4N - 4)/(V_k + 2)$. Concomitantly, genetic diversity maintained by such a species at equilibrium should be much less than that expected on the basis of sheer abundance. Thus, this hypothesis makes three testable predictions: (i) lower genetic diversities (particularly of mitochondrial haplotypes) within cohorts of larvae or newly recruited juveniles than exists in the adult spawning population, (ii) significant genetic diversity among cohorts of larvae or recruits, and (iii) temporal genetic variance owing to random drift in finite populations. The last prediction is supported by measurements of genetic drift in a semi-isolated population of Pacific oyters *Crassostrea gigas* in Dabob Bay, WA, for which a 30-year record of larval production and recruitment success is available (Hedgecock 1994). Here, estimated $N_e \approx 400$ contrasts with annual harvests of at least 10^7 oysters. These predictions are being quantified by computer simulations, which also permit investigation of the statistical properties of genetic parameters such as drift variance and various estimators of N_e. Empirical testing involves comparisons of genetic diversities among samples of larvae, juveniles, and adults from the Dabob Bay oyster population. Genetic studies of larval and spat populations are made possible by enzymatic amplification of target DNA sequences from individual larvae by the polymerase chain reaction (PCR). PCR products of mitochondrial DNA are being screened for single-stranded conformational polymorphisms (SSCP) and restriction fragment length polymorphisms (RFLP). Microsatellite loci are also being developed in order to measure gametic phase disequilibria, which are expected to be non-zero in larval cohorts produced by a limited number of parents.

Population Structure II

B9-017 THE ISTHMUS OF PANAMA, MOLECULAR CLOCKS AND FISH BIOGEOGRAPHY, Eldredge Bermingham, Andrew Martin, and Shawn McCafferty, Smithsonian Tropical Research Institute, Apartado 2072, República de Panamá.

The rise of the isthmus of Panama separated neotropical marine habitats and joined terrestrial ones. For several years we have studied the influence of this recent earth history event on molecular evolution in marine fish populations split by the rising isthmus and in freshwater fish populations invading a new terrestrial landscape. We have measured protein divergence by electrophoresis and mitochondrial DNA differentiation by direct sequence analysis for 20 species pairs of fish separated by the Panamanian isthmus. By using data from two classes of macromolecules, we are better able to distinguish between dissimilarities arising from different dates of separation and dissimilarities that may be due to different rates of evolution. Because many of the transisthmian species pairs are representatives of circumtropically distributed species groups, our transisthmian fish data also provide a logical jumping-off point for historical biogeographic analyses of tropical marine fishes. We will exemplify this approach with our molecular systematic studies of the Abudefduf saxatilis and A. sordidus species complexes. In addition, our marine fish data provide fruitful comparisons to our studies of the historical biogeography of Panama, a landscape thought to have been colonized by freshwater fishes over the past three million years. In turn, we will argue that molecule-based, historical biogeographic analyses of tropical faunas and floras provide the most efficacious means of determining whether species richness is principally controlled by regional or local processes and for identifying priority areas for biodiversity conservation.

B9-018 DIRECT EVIDENCE ABOUT BOTTLENECKS IN MARINE ORGANISMS: THE 1983 *DIADEMA* PANDEMIC, H.A. Lessios, Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama.

Though a great deal of theoretical and experimental evidence suggests that genetic bottlenecks are important in the evolution of populations, there has never been an opportunity to study their effects directly in a natural population. The 1983 epidemic that reduced populations of the sea urchin *Diadema antillarum* by >95% throughout the neotropics is the first event that makes such a study possible. Isozyme data of Panamanian populations of this species, taken in 1976-1977, and exhaustive documentation of total population size permit an assessment of the degree to which the mass mortality caused changes in genetic variability and gene frequencies. The same two populations that had been studied in 1976-1977 have been sampled by the same techniques each year from 1983 to 1994. Two additional sea urchin species, *Echinometra lucunter* and *E. viridis*, which did not suffer mass mortality, have also been followed, so that they can act as controls for the possibility that changes seen in *D. antillarum* were caused by factors other than population bottlenecks. Eleven years after the mass mortality, populations of *D. antillarum* are still at less than 3.5% of pre-1983 densities. Yet, despite this reduction in total population size over ten generations, heterozygosity and number of effective alleles have remained constant. Significant gene frequency changes were observed in some loci of both *Diadema* and the control species, so they are more likely to have been caused by vagaries of larval settlement. The results suggest that marine species with planktonic larvae are, on the genetic level, resilient to severe perturbations in their population size. This finding has theoretical importance in the reconstruction of factors involved in the evolution of such organisms and the management of threatened coral reef organisms.

B9-019 **INFERRING POPULATION HISTORY FROM MOLECULAR PHYLOGENIES, Sean Nee, Eddie Holmes, and Paul H. Harvey, Department of Zoology, South Parks Road, Oxford, OX1 3PS, United Kingdom.**

Gene sequence information opens a new window on the dynamical history of a population. A phylogenetic tree of a sample of sequences from a population contains in its structure information about the population's past. A molecular phylogeny with a temporal dimension, provided by a molecular clock, is required, although it is not necessary that the clock is calibrated. Given such a phylogeny, techniques, based on coalescence theory from population genetics, are available to make inferences about the populations past. One may infer, for example, whether or not the population has been growing or declining and how it has been growing or declining. Examples of the application of these techniques include humpback whales, Hepatitis C and Dengue fever virus.

B9-020 DNA POLYMERASE GENES AS PROBES OF THE DIVERSITY AND PHYLOGENY OF MARINE MICROBIAL POPULATIONS, Curtis A. Suttle, Feng Chen and Matthew T. Cottrell, Marine Science Institute, University of Texas at Austin, PO Box 1267, Port Aransas TX 78373.

Marine viruses are possibly the most abundant biological entities on earth while bacteria constitute the greatest biomass in the oceans. Despite the ecological importance of marine bacteria and viruses to marine ecosystems and the biosphere, we have only begun to scratch the surface in terms of the structure, diversity and evolutionary relationships among these components. One approach for trying to measure diversity and genetic relatedness among components in marine microbial communities is by sequence analysis of DNA polymerase genes. DNA polymerase genes are excellent candidates for examining genetic and phyletic relationships because they possess highly conserved and variable domains, and occur in all living organisms as well as in many types of viruses. A significant concern when estimating genetic relatedness based on sequence analysis is that differences inferred from a single gene may not accurately reflect the genetic distance among entire genomes. We have examined this question using total genomic DNA hybridization and sequence analysis of DNA polymerase genes to compare genetic similarities among viruses (*Mp*V) which infect a marine photosynthetic flagellate, *Micromonas pusilla*. These are large double-stranded DNA viruses that are ubiquitous in distribution and which can occur at abundances of $>10^5$ ml^{-1} in natural waters. We examined 8 clonal isolates of *Mp*V obtained from widely separated geographic locations, including the coastal waters of New York, Texas, California and British Columbia, and the central Gulf of Mexico. Restriction fragment digests indicated that each of these clones was genetically different (1). Analysis of genetic relatedness among these viruses using either hybridization of total genomic DNA or sequence analysis of DNA polymerase genes demonstrated that the isolates belonged to a group of closely related viruses, and that the genetic variation among *Mp*V isolated from the same water sample can be as large as the variation among viruses isolated from different oceans. Moreover, sequence analysis showed that viruses which infect *M. pusilla* are related to morphologically similar viruses which infect other photosynthetic microalgae, namely, *Chrysochromulina* spp. and *Chlorella* spp. The hybridization and sequencing methods resulted in similarities between all pair-wise combinations that were well correlated ($r = 0.90$). These results indicate that DNA polymerase genes provide a good basis for examining the genetic structure and diversity of marine microbial populations. The approach should also be suitable for examining populations of marine bacteria and other organisms.

Cottrell, M.T. and C.A. Suttle, 1991. Wide-spread occurrence and clonal variation in viruses which cause lysis of a cosmopolitan, eukaryotic marine phytoplankter, *Micromonas pusilla*. *Mar. Ecol. Prog. Ser.* **78**:1-9.

Adaptation to the Environment

B9-021 THE ECOLOGICAL SIGNIFICANCE OF GENETIC DIVERSITY IN CORALS AND THEIR ALGAL SYMBIONTS, Rob Rowan[1,2] and Nancy Knowlton[2], [1]Australian Institute of Marine Science, Townsville, Australia, [2]Smithsonian Tropical Research Institute, Balboa, Republic of Panama.

The assumption of substantial morphological plasticity underlies much of the traditional taxonomy of marine invertebrates and has led to the prevailing view that many species have broad ecological requirements. Recent genetic analyses suggest, however, that many so-called species are really complexes of sibling species, each with narrower habitat or biogeographic distributions. For example, the dominant Caribbean coral, *Montastraea annularis* sensu lato is now known to consist of at least three species: *M. annularis* sensu stricto, *M. faveolata* and *M. franksi*. These species differ in their depth distributions and exhibit a suite of differences in colony morphology and life history that appears to be related to the different intensities of wave energy they characteristically experience. Like other reef-building corals, members of the *M. annularis* complex form obligate associations with photosynthetic microalgal endosymbionts called zooxanthellae. Genetic analyses reveal considerable diversity within these symbionts as well. Both *M. annularis* and *M. faveolata* associate with representatives of the three distinct types of zooxanthellae previously reported from other hosts. Within the depth range inhabited by these two corals, the three zooxanthella types exhibit clear depth zonation. *Montastraea franksi*, which does not generally occur in very shallow water, hosts the zooxanthella type found in deeper water specimens of the other two coral species. These patterns suggest ecological specialization by the different types of zooxanthellae for different light regimes. Individual samples from both *M. annularis* and *M. faveolata* often appear to contain two types of zooxanthellae. In some colonies of *M. annularis*, the two taxa of symbionts can vary in their proportions across the colony, but additional sampling is needed to determine if predictable patterns of distribution occur within colonies. Polymorphic, habitat-specific symbioses may permit distributions of hosts and symbionts to respond independently to different environmental gradients, thereby challenging the conventional view that the host is the fundamental unit of biodiversity. Many distinctive aspects of marine animal-algal associations are also illuminated by these findings. Habitat specificity provides ecological explanations for the previously documented poor concordance between host and symbiont phylogenies and the otherwise surprising lack of direct, maternal transmission of symbionts in many species of hosts. Polymorphic symbioses may underlie the conspicuous and enigmatic variability characteristic of responses to environmental stress (e.g. coral "bleaching") and contribute importantly to the phenomenon of photoadaptation.

B9-022 A MULTIDISCIPLINARY APPROACH TOWARD RESOLVING THE SELECTIONIST/NEUTRALIST CONTROVERSY,

Dennis A. Powers[1], Leonard DiMichele[2], Douglas Crawford[3], Patricia Schulte[1], Giacomo Bernardi [4], [1]Department of Biological Sciences, The Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950, [2]Wildlife & Fisheries Sciences, Texas A&M University, College Station, TX 77843, [3] Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL. 60637, [4]University of California, Santa Cruz, CA.

Using the model teleost *Fundulus heteroclitus*, we have been employing an experimentally-based strategy to address the "selectionist/neutralist" controversy. In an attempt to falsify the hypothesis that natural selection was (or is) a major driving force responsible for the observed gene diversity in this species, we have been using a multidisciplinary approach. The first step was to establish the biogeography in relation to environmental variables. We used gel electrophoresis for detecting allelic isozymes, restriction fragment length polymorphisms (RFLP) for evaluating mtDNA haplotypes, and DNA sequencing to determine allelic alternatives for selected genomic and mitochondrial loci. We have studied over 50 protein-encoding loci and found that approximately half show some degree of genetic variation. Based on the biogeographical information, our experimental strategy began with a detailed biochemical study of the kinetics and thermodynamics of allelic isozyme variants in relation to temperature and pH, then we progressed to higher levels of biological organization by a linked series of predictions followed by experimental validation. We found evidence for differences in enzyme concentration, thermal stability, and/or steady-state kinetics between allelic isozymes of the following seven loci: the lactate dehydrogenase "heart" locus (*Ldh-B*), a cytoplasmic malate dehydrogenase locus (*Mdh-A*), a cytoplasmic NADP-dependent isocitrate dehydrogenase (*Idh-B*), hexose-6-phosphate dehydrogenase (*H6pdh-A*), a glucose phosphate isomerase locus (*Gpi-B*), a phosphoglucomutase locus (*Pgm-B*), and a cytoplasmic aspartate amino transferase locus (*Aat-A*). These results led us to make predictions about differential gene regulation, gene expression, amino acid differences between allelic isozymes, allele specific differences in metabolic flux and carbon partitioning, and organismic performance differences between allotypes, as well as estimates of differential fitness. Our predictions were validated as we established differences in: the concentrations of allelic isozyme, allele specific amino acid replacements, locus specific mRNA levels, transcription rates, DNA sequences of 5'-regulatory regions, DNA footprinting, expression in tissue culture, on-going transgenic fish studies, cellular metabolism, genotype specific swimming ability, developmental rates, hatching times, and laboratory and field selection experiments. Since the work on *Ldh-B* has been most extensive to date, we will illustrate our strategy by elaborating on the allelic alternatives of the *Ldh-B* locus in order to adequately demonstrate the extent of our success with an interdisciplinary experimental approach toward resolving the neutralist/selectionist controversy.

Late Abstracts

THE GENETIC STRUCTURE OF NATURAL POPULATIONS OF THE COPEPOD *TIGRIOPUS CALIFORNICUS* AND ITS EVOLUTIONARY CONSEQUENCES, Ronald S. Burton, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0202.

Populations of the harpacticoid copepod *Tigriopus californicus* inhabit high intertidal pools along the Pacific coast of North America. Allozyme studies have shown that neighboring conspecific populations frequently harbor unique alleles at high frequency, indicating highly restricted present day gene flow. More remarkable is the level of population differentiation at the DNA level, with a recent study (1) showing 18% sequence divergence at the COI locus (mtDNA) between populations from central (Morro Bay to San Francisco) and southern California (Los Angeles to San Diego). Nuclear DNA sequence variation (5" end of a histone H1 gene) was completely concordant with the mtDNA differentiation, showing sharp regional differences between central and southern populations. Because this phylogeographic break occurs in the region of Pt. Conception, a well documented biogeographic boundary, additional work on genetic structure has focused on the transition zone, including two coastal populations (Carrillo Beach and Pt. Dume) and several populations from the Channel Islands. Results to date show that although allozyme differentiation between the coastal sites and previously studied Los Angeles and Morro Bay populations is negligible, sequence differentiation (both nucDNA and mtDNA) sets these populations apart as a third clade only distantly related to the previously identified central and southern California clades. Samples from the north and south sides of Santa Cruz Island not only show remarkable divergence from all mainland populations but are also strikingly divergent themselves: comparison of COI sequences from the north and south sides of the island revealed that 24 of 32 possible third base substitutions had occurred. These results clearly demonstrate a history of population isolation probably on the order of millions of generations. Despite this unprecedented level of genetic differentiation, *T. californicus* populations retain interfertility. Assessment of the evolutionary consequences of population differentiation has relied on analysis of laboratory hybrids between natural populations. These studies have documented a consistent pattern of F_2 hybrid breakdown suggestive of pervasive genomic coadaptation. F_2 interpopulation hybrids develop more slowly and sometimes lack elements of physiological response common to both parental populations. The genetic basis of common physiological processes has, therefore, differentiated among natural populations, ultimately leading to outbreeding depression. With respect to response to salinity stress, a biochemical pathway induced by osmotic stress in natural populations fails to function in some F_2 interpopulation hybrids. Molecular analysis of this system may provide insight into the evolution of adaptation and genomic coadaptation.

(1) Burton, R. S. and B.-N. Lee (1994) Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. Proc. Nat. Acad. Sci. USA. 91:5197-5201.

Molecular Approaches to Marine Ecology and Evolution

LONG-DISTANCE DISPERSAL OF LARVAL MARINE FISH, Robert K. Cowen[1] and Jonathan A. Hare[2]. [1]Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, [2]Southeast Fisheries Science Center, Beaufort Laboratory, 101 Pivers Island Rd, Beaufort, NC 28516

The mechanisms of larval dispersal are critical to population dynamics and community structure in marine systems. Understanding these mechanisms involves detailed knowledge of the larval ecology of the species (defined broadly to include egg and larval stages, transition from larva to juvenile, and settlement) and how the ecology of the planktonic stages interacts with the physical oceanography of the region under study. In our work examining larval transport, we frequently encounter constraints presented by traditional analytical techniques and have started to explore the utility of molecular techniques. In many ichthyoplankton studies, larvae are identified only to the family level because of imprecision in lower taxonomic determinations. This identification constraint complicates the study of larval transport and our ability to draw conclusions about the role of transport in recruitment variation. Molecular techniques, however, can be used to identify larval types to species allowing finer taxonomic resolution. Yet, identification to species is itself limiting to certain questions. To the extent that distinct populations exist, species-level identifications constrain the resolution of population level questions. Again, molecular techniques offer a solution. Through the identification of population-specific genetic and morphological markers, larvae can be traced from their source populations and the larval contribution from different populations can be determined at a specific site. Finally, molecular techniques allow phylogenetic hypotheses to be developed regarding the evolution of larval traits (e.g. larval duration, age and size at metamorphosis) that potentially are important to larval transport. Thus, the advent of molecular techniques adds to the interdisciplinary tools required in the study of larval transport of marine fish, enabling a finer resolution of both questions and answers.

THE FOSSIL RECORD OF SPECIATION IN THE SEA, Jeremy B. C. Jackson, Center for Tropical Paleoecology and Archaeology, Smithsonian Tropical Research Institute, Box 2072 Balboa, Republic of Panama.

The great majority of species of planktonic foraminifera, corals, mollusks, bryozoans, and other marine taxa with a good fossil record appear abruptly and persist unchanged morphologically for millions of years. Moreover, this punctuated pattern is clearly not a stratigraphic artifact in the few cases with good morphometric definition of species, detailed sampling, and tight confidence intervals for the timing of first and last appearances of species. In some cases, there is even excellent correspondence between species defined morphometrically and genetically. Speciation and extinction also commonly occur in pulses, so that half or more of the entire fauna turns over in only a few hundred thousand years, compared to intervening periods of many millions of years duration with very low rates of speciation and extinction. These turnover "events" are often well correlated with major global climatic change which may also be quite sudden. The last major turnover occurred about 2 million years ago, with very little subsequent speciation or extinction. The implication is that population genetic structure studied by biologists is background noise important to local adaptations, but largely irrelevant to speciation and extinction most of the time; a pattern consistent with Sewall Wright's Shifting Balance Theory. It is difficult, however, to reconcile these paleontological results with the common assumption of a constant molecular evolutionary clock, at least for those clades in which morphology and molecules are tightly correlated.

PHYSIOLOGICAL MOLECULAR CLOCKS: EVIDENCE, IMPLICATIONS AND PREDICTIONS. Andrew P. Martin, Dept. of Biological Sciences, University of Nevada-Las Vegas, Las Vegas, NV 89154.

Identifying factors that influence rates and patterns of nucleotide substitution is important for understanding the molecular basis of mutation, developing models of DNA evolution, inferring phylogenetic relationships, and estimating demographic parameters from population genetic data. In this talk, variation in nucleotide substitution rates among lineages is reviewed and related to potential causative factors. The concept of nucleotide generation time is introduced, which is defined as the amount of time needed for a given site to be copied, either by replication or repair. Comparison of DNA substitution rates in physiologically-different animals with similar generation times (i.e. sharks and mammals) suggests that metabolic rate may play a large role in setting nucleotide generation times, and therefore the pace of DNA evolution. This hypothesis has important implications because it suggests physiological characteristics of organisms may influence rates of DNA evolution, and substitution rate heterogeneity among lineages will probably be rampant in taxa with markedly different specific metabolic rates. Predictions of hypotheses of molecular rate variation can be cast in terms of defining factors which set nucleotide generation times. Possible tests of alternative hypotheses are outlined.

EVOLUTION OF GAMETE RECOGNITION AND THE SPECIATION PROCESS, Stephen R. Palumbi and Edward C. Metz, Department of Zoology and Kewalo Marine Laboratory, University of Hawaii, Honolulu, HI.

Fertilization barriers between closely related species furnish the opportunity to examine the molecular evolution and population genetics of reproductive isolation. We sequenced coding regions for the sperm-egg attachment and fusion protein bindin from three closely related sea urchins that show strong blocks to cross-fertilization. Sequences from 39 individuals show strong species specific differences, and also demonstrate the existence of divergent alleles within all three species. Phylogenetic analysis shows an excess of amino acid replacements relative to silent substitutions in a highly variable portion of the bindin coding sequence. Furthermore, an excess of amino acid replacements in this region are non-conservative. These patterns occur in comparisons between species as well as between divergent alleles within species. Thus, much of the intra- and inter-specific variation in bindin results from diversifying selection and is likely to reflect functional polymorphism within and between species. These results show that the genetic variation required for rapid evolution of sexual isolation can be found in natural populations.

THE ROLE OF THE *EUPRYMNA SCOLOPES* LIGHT ORGAN SYMBIOSIS IN THE ECOLOGY AND EVOLUTION OF *VIBRIO FISCHERI*, Edward G. Ruby, Department of Biological Sciences, University of Southern California, Los Angeles.

Symbiotic interactions between bacteria and their animal and plant hosts have been shown to play a major role in the ecology and evolution of many terrestrial organisms. While marine symbioses must have a similar impact, an examination of the ecological effects of these associations, and the mechanisms underlying them, has been hindered by the complexity of aquatic environments. The light organ symbiosis between the Hawaiian sepiolid squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri* provides an emerging model system for discovering the scope of this association's role in the ecology and population genetics of the animal and bacterial partners. Using molecular probes specific for *V. fischeri* we have shown that the abundance of these bacteria in Hawaiian seawater is directly connected to the presence of symbiotic animals, which expel excess symbionts from their light organs into the surrounding environment on a diel basis. It is these bacteria that are responsible for the subsequent inoculation of the developing light organs of the next generation of newly hatched *E. scolopes*. Analyses using polymerase chain reaction (PCR) amplification have revealed that the majority of *V. fischeri* cells in the seawater of these habitats exist in a nonculturable, but symbiotically infective, state. These dormant cells account for over 99% of the *V. fischeri* detectable in Hawaiian seawater, and may be poised specifically to colonize the nascent light organ of juvenile squids. The population structure of isolates of *V. fischeri* from Hawaiian seawater consists of two groups (or clades) that can be distinguished by sequence divergences at 6 known genetic loci. Symbionts isolated from the light organs of *E. scolopes* consistently fall into only one of these clades, and they are more closely related to symbiotic *V. fischeri* isolated from Japanese sepiolid squids than to the other, apparently nonsymbiotic, clade of Hawaiian *V. fischeri*.

The process of light organ colonization by *V. fischeri* is easily studied by exposing newly hatched squids to specific strains of *V. fischeri* and experimentally following the initiation and subsequent persistence of the symbiotic infection. Competition studies performed by co-inoculating light organs with representatives of the two clades of Hawaiian *V. fischeri* isolates have revealed that the symbiotic clade is consistently dominant after the first 24 hours, and that this competitive edge may result from a differential ability to compete for host-supplied nutrients. To determine the genetic identity of this and other bacterial traits required for symbiotic competency, we have isolated transposon-induced mutants of *V. fischeri* that are compromised in the process of symbiotic colonization at specific stages in the development of the association. These mutants have allowed us to begin to describe the microecology of the bacteral-squid interaction within the light organ.

MEASURING POPULATION SUBDIVISION IN MARINE SPECIES: THE IMPORTANCE OF SPATIAL SCALE, Stephen M. Shuster, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ U.S.A.

The first phase of Wright's Shifting Balance process involves the fragmentation of populations into semi-isolated subgroups that become genetically distinct by drift. Wright developed his "F-statistics" to quantify the degree to which this phase has occurred, and as genetic data become more accessible, this and related methods are increasingly used to document population subdivision in marine taxa. However, meaningful interpretation of F-statistics requires knowledge of the spatial scale at which random mating occurs, data usually unavailable without detailed life history information on the organisms investigated. Since tests for nonrandom mating can be robust, I attempted to determine if incorrect estimates of the spatial scale at which random mating occurs can generate misleading estimates of population subdivision. F-statistics calculated for simulated populations with known genetic substructure show that such mistakes can occur without violating Hardy-Weinberg expectations. Moreover, these results show that the relative values of F_{IS}, F_{IT} and F_{ST} provide a characteristic signature when the scale of analysis, i.e., how subdivisions are recognized, matches or does not match the actual breeding structure of the population. Population structure within subpopulations due to selfing, close inbreeding or demersal larvae can generate apparently unstructured populations that are in fact highly subdivided. These considerations are often overlooked in current research on the genetic structure of marine populations.

Poster Session

B9-100 Major genetic differentiation of Crown-of-thorns starfish populations in the Indian Ocean from those in the Pacific. John A. H. Benzie, Australian Institute of Marine Science, PMB 3, Townsville QLD 4810, Australia

Crown-of-thorns starfish (Acanthaster planci) populations show little genetic differentiation throughout the Pacific. Relatively isolated populations, such as Lord Howe Island and Hawaii, demonstrate some shift in gene frequencies, probably as a result in part of founder effect as well as the lack of gene flow from other populations. Recent surveys of genetic variation in populations of Crown-of-thorns starfish from several sites in the Indian Ocean show marked shifts in gene frequency at many loci. Although no fixed gene differences were found, close examination of populations in the Indo-Malay region will be required to establish whether there is gene exchange between Indian Ocean and Pacific types or whether the Indian Ocean Crown-of-thorns is a new species.

B9-101 GAMETIC COMPATIBILITY AND HYBRIDIZATION OF SYMPATRIC PATIRIELLA SPECIES (ECHINODERMATA: ASTEROIDEA) IN NEW SOUTH WALES, Maria Byrne, Marti Jane Anderson, Department of Anatomy and Histology, F-13, University of Sydney, Sydney, New South Wales, 2006, Australia

Three species of the asteroid genus Patiriella occur sympatrically in New South Wales and the possibility for hybridization between them was examined through a series of cross-fertilization experiments. P. calcar and P. gunnii are morphologically distinct as adults, but indistinguishable as larvae. P. exigua is morphologically distinct in both its adult and larval morphologies. The gametes of P. calcar and P. gunnii were reciprocally compatible: laboratory crosses between these species produced viable hybrid juveniles. In crosses between female P. calcar and male P. gunnii, most of the juveniles metamorphosed with an arm number intermediate between that of the parents, whereas crosses between female P. gunnii and male P. calcar produced juveniles with an arm number more similar to the maternal phenotype. Heterospecific crosses with P. exigua resulted in low fertilization rates and viable hybrids were not produced. This species appears to be capable of self-fertilization. Because hybrids between P. calcar and P. gunnii were viable, neither gametic incompatibility nor hybrid inviability appear to ensure reproductive isolation between these species. Ecological or habitat segregation and temporal separation in breeding may isolate these species in the field. The results demonstrate that if gamete surface recognition molecules are involved in fertilization of P. calcar and P. gunnii, then they are not strongly species-specific, at least at the sperm concentrations used in this study. Reproductive isolation between these species has evolved despite their gametic compatibility. In contrast, P. exigua is isolated from its congeners due to gametic incompatibility and several features characteristic of its reproduction and development. The implications of these findings for reproductive isolation and speciation of Patiriella and for the evolution of reproductive isolation in free-spawning marine organisms are discussed.

B9-102 SYSTEMATIC RELATIONSHIPS BETWEEN TROPICAL CORALLIMORPHARIANS (ANTHOZOA: CORALLIMORPHARIA): UTILITY OF THE 5.8S AND INTERNAL TRANSCRIBED SPACER (ITS) REGIONS OF THE rRNA TRANSCRIPTION UNIT. Chaolun A. Chen[1,2], Bette L. Willis[1], David J. Miller[2]. [1]Department of Marine Biology, [2]Department of Molecular Sciences, James Cook University of North Queensland, Townsville, Queensland 4811, Australia.

The nucleotide sequences of a segment of the rRNA transcription unit spanning the 3'-end of the 18S rDNA to the 5'-end of the 28S rDNA were determined for the tropical corallimorpharians (Cnidaria: Anthozoa), Rhodactis howesii, R. mussoides, Amplexidiscus fenestrafer, Actinodiscus nummiformis, A. unguja and an undescribed species. Comparison of the 5.8S rDNA sequences indicated a closer relationship between the genera Rhodactis and Amplexidiscus than between the former and Actinodiscus. However, the level of observed variation in this conserved region of the ribosomal transcription unit is not consistent with den Hartog's revision of the family Actinodiscidae to a single genus. Amongst the range of species studied, there was considerable varation in both length and %(G+C) content in the ITS regions. Both ITS sequences appear to be conserved within genera but highly variable between genera, and can therefore be used for generic assignment. Analysis of both ITS and 5.8S rRNA sequences support assignment of the undescribed species to the genus Amplexidiscus.

B9-103 ECDYSTEROID RECEPTOR GENE EXPRESSION DURING LIMB REGENERATION IN THE CRAB, *UCA PUGILATOR*, David S. Durica, Penny M. Hopkins and Arthur Chung, Department of Zoology, University of Oklahoma, Norman OK 73019

Growth in arthropods is limited by their rigid exoskeleton. Unlike insects, most crustaceans continue to grow throughout the adult stage, via molting cycles that are mediated by ecdysteroid hormones. This increase in general body dimensions, however, can also be accompanied by another specialized type of growth that results in the regeneration of lost or damaged limbs. The factors involved in coordinating growth in regenerating animals is unclear. We have examined of the role of ecdysteroids in the regeneration process. Although ecdysteroids are involved in mediating hypertrophic growth during proecdysial limb regeneration, a potential role in the developing limb primordium has not been characterized. We have recently initiated an examination of the role of ecdysteroids in the early regeneration process and are interested in exploring relationships between variations in ecdysteroid hormone titers during the molt cycle and hormone receptor expression in regenerating tissues.

Using sequence information derived from the *Drosophila* ecdysteroid receptor (EcR) gene, we have isolated a crab cDNA clone containing the *Uca* EcR DNA binding domain. The nucleotide sequence is 76% identical to the *Drosophila* EcR DNA binding domain, and encodes six amino acid substitutions, clustered within the second zinc finger. The gene appears to be single copy in the *Uca* genome, and in contrast to *Drosophila*, contains an intron within the DNA binding domain. RT-PCR, Western blot analysis and native gel binding assays indicate that the ecdysteroid receptor is expressed in early limb bud primordia. Experiments are currently underway to monitor EcR transcript levels in staged regenerating limb bud and epidermal tissue and to examine potential co-expression with members of the retinoid-like family of receptors, which form hetero-dimers with EcR to regulate transcription in *Drosophila*.

B9-104 RAPD'S AS NUCLEAR GENE MARKERS OF POPULATION STRUCTURE AND HYBRIDIZATION IN LAKE VICTORIA CICHLIDS, Paul A. Fuerst, Wilson Mwanja, Gregory Booton, Melissa Black, Mark Chandler* and Les Kaufman**, Depts. of Molecular Genetics and Zoology, The Ohio State University, Columbus, OH 43210, *New England Aquarium, Boston, MA 02110 and **Dept. of Biology, Boston University, Boston, MA 02215

The cichlid fauna of Lake Victoria has undergone significant loss and continued endangerment after the introduction of an exotic predator, the Nile Perch, *Lates niloticus*, and an ecologically labile exotic cichlid competitor, the Nile tilapia, *Oreochromis niloticus*. Among species severely affected by the introductions was the Ngege, *O. esculentus*, one of two endemic L. Victoria tilapias. Ngege have been extirpated from L. Victoria, and survive only as remnant populations in several small satellite lakes. We have used molecular methods, including RAPD markers, to examine population structure of some remaining populations of Ngege. Populations of Ngege from Lakes Nabugabo, Manywa, Kachira, Mburo, and Kanyoboli, and congeners from these lakes and from Lakes Victoria, Albert and George, were studied. Genetic differences in RAPD patterns between individuals within populations are small, but differentiation between populations is manifest. Hybridization between *O. esculentus* and other sympatric congeners, including *O. niloticus* and *O. leucostictus*, is estimated. The studies of a haplochromine species, *Astatoreochromis alluaudi*, are contrasted with the results from *Oreochromis*. *A. alluaudi* remains widespread, both in L. Victoria and in surrounding rivers and lakes. Populations of *A. alluaudi* appear to be less differentiated than sympatric Ngege. As expected, population differentiation for either wisespread species is less than interspecies differences between twenty very closely related narrow endemic L. Victoria haplochromine species. For both tilapine and haplochromine species, RAPDs are a useful tool to identify population and species differences, even when mt-DNA suggests little or no population differentiation. (Supported by the National Science Foundation and the Pew Charitable Trust).

B9-105 RAPID SCREENING OF PCR PRODUCTS FOR ANALYSIS OF POPULATION STRUCTURE, Patrick M. Gaffney, College of Marine Studies, University of Delaware, Lewes, DE 19958

Direct sequencing of PCR products is an increasingly popular method of detecting sequence variants for various uses in ecology and evolution, including the analysis of population structure. In cases where one or a few sequence variants predominate, sequencing large numbers of individuals provides relatively little information for the effort expended. If identical sequences can be identified in advance, redundant sequencing can be avoided. After denaturation and reannealing, pairwise combinations of PCR products can be screened for heteroduplex formation by several methods, including conformation-sensitive gel electrophoresis and S1 endonuclease digestion. Heteroduplex formation indicates sequence non-identity. A matrix of pairwise combinations can be thus analyzed to collapse a set of PCR products into a smaller number of unique sequences suitable for further analysis. A reference set of sequenced PCR products can be used to screen new products. This procedure can be used to identify amplified regions with desirable levels of sequence diversity as well as for identifying individuals with specific sequences. Applications of this method to the study of population structure in marine bivalves and finfish are illustrated.

B9-106 ^{31}P NUCLEAR MAGNETIC RESONANCE MEMBRANE PHOSPHOLIPID PROFILING USED TO DIFFERENTIATE AMONG THREE LAKE MICHIGAN SPONGES OF THE GENUS *Spongilla*, Thomas Glonek[1], Lidia G. Bardygula-Nonn[2], Timothy A. Early[3], [1]MR Lab., Midwestern U., Chicago, IL 60615; [2]Center For Great Lakes Studies, U. Wisconsin-Milwaukee, Milwaukee, WI 53204; [3]Aquatic Resource Center, Hammond, IN 46320

With the explosive growth of zebra mussels in the Southern basin of Lake Michigan, three morphologically-distinct sponges, rounded, which overgrows and kills zebra mussels, encrusting, and fingerform, also have undergone exponential growth. Similarity and degree of relatedness among the three sponge forms has initiated controversy. We quantified sponge phospholipid compositions (Glonek, in Quin and Verkade, P-31 NMR Spec. Prop. in Com. Chara. & Struct. Anal., VCH Pub., NY, 1994), reasoning that the cellular membrane composition reflects, at the molecular level, the phenotypic expression of sponge genome. Respective rounded, encrusting, and fingerform phospholipid compositions are (mole %: Statistics; Oneway; Scheffé range $P < 0.05$; n = 8; a, rounded from encrusting; b, rounded from fingerform; c, encrusting from fingerform): lysophosphatidic acid, 1.87, 2.13, 3.12^{b,c}; glycerol plasmalogen, 0.87, 0.84, 0.92; phosphatidylglycerol, 0.71, 0.31^{a}, 0.31^{b}; lysoethanolamine plasmalogen, 1.13, 0.74^{a}, 0.74^{b}; lysophosphatidylethanolamine, 0.63, 1.02^{a}, 0.89; phosphatidic acid, 0.07, 0.14, 0.05; alkylacylphosphatidylethanolamine, 0.44, 1.02^{a}, 1,34^{b}; diphosphatidylglycerol, 3.66, 4.60, 4.07; dihydrosphingomyelin, 1.98, 1,60, 2.35^{c}; ethanolamine plasmalogen, 21.10, 19.66, 20.32; phosphatidylethanolamine, 14.98, 14.87, 13.72^{b,c}; phosphatidylserine, 11.60, 11.44, 10.41^{b,c}; uncharacterized phospholipid, 0.64, 0.55, 0.60; lysoalkylacylphosphatidylcholine, 3.38, 3,19, 2.49^{b,c}; lysophosphatidylcholine, 1.48, 1,24^{a}, 1,25^{b}; phosphatidylinositol, 3,29, 3,62, 3,22^{c}; alkylacylphosphatidylcholine, 20.82, 22.70^{a}, 21,76^{b,c}; phosphatidylcholine, 10.76, 9.62^{a}, 11.35^{c}; diacylglyceryl-(2-aminoethyl)phosphonates, 0.63, 0.73, 1.09^{b,c}. All three sponge forms contain the same phospholipids; however, the three forms exhibit statistically different phospholipid concentrations in 14 of the 19 phospholipids detected.

B9-107 POPULATION GENETICS AND GENEALOGY OF A COSMOPOLITAN AND INVASIVE SPECIES

Carol E. Lee, School of Oceanography WB-10, University of Washington, Seattle, WA 98195

Eurytemora affinis is a cosmopolitan copepod found in coastal seas, estuaries, rivers, lakes, and ponds throughout northern latitudes of North America, Europe and Asia. Its wide distribution results in part from the ease with which it can colonize new habitats, either by 'natural' dispersal mechanisms, or by anthropogenic mechanisms such as transport by ship ballast water. The ability to tolerate a broad range in temperature and salinity, may allow *E. affinis* to enter new habitats, become established, and perhaps experience selection. Examination of genetic structure using allozyme and DNA markers showed genetic distinctiveness among geographically distant and adjacent estuarine populations.

B9-108 USE OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) FOR SPECIES IDENTIFICATION OF LARVAE AND QUANTIFICATION OF GENETIC VARIABILITY TO ANALYSE DISPERSAL PATTERNS IN TWO CONGENERIC BIVALVE SPECIES, Mats Lindegarth, Carl André, Per Jonsson, Tjärnö Marine Biological Laboratory, S-452 96 Strömstad, SWEDEN, Per Sundberg, Department of Zoology, 413 90 Göteborg, SWEDEN

Two congeneric bivalve species, Cerastoderma edule and C. lamarcki, both vary in abundance and age structure at spatial scales ranging from 1 m to 10 km. Moreover, the two species differ in habitat preferences. Several models may be proposed as responsible for the observed patterns, many of which are connected to the larval phase lasting for approximately 2 weeks. However, the great difficulty involved in identifying the species of larvae and juveniles poses a major problem in testing hypotheses about recruitment processes. As a tool in experimental and descriptive work, attempting to identify important processes, we have developed a technique to determine species identity of individual larvae using banding patterns of RAPD-fragments. Banding patterns of laboratory reared larvae of both species have been compared with adult individuals collected from several sites on the Swedish west coast. Out of 16 screened random primers, several diagnostic banding patterns have been identified. The molecular studies also reveal intraspecific banding polymorphisms which may be used to quantify the extent of genetic divergence among sub-populations. Coupled to computer models, this technique will be used to infer potential population genetic effects of variability in larval dispersal caused by varying shoreline complexity.

B9-109 MITOCHONDRIAL DNA DOMAINS FOR PCR DETECTION AND THE PHYLOGENETIC CHARACTERIZATION OF THE OYSTER PROTOZOAN PARASITE *PERKINSUS MARINUS*. Adam G. Marsh[1], Giacomo Bernardi[2] and Gerardo R. Vasta[1], [1]Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD 21202; [2]Dept. of Biology, University of California, Santa Cruz, CA 95064.

The parasite *Perkinsus marinus* is the cause of severe mortalities in the eastern oyster, *Crassostrea virginica*. We have sequenced 8.5 Kbp of the *P. marinus* mt-genome and have identified several non-coding intergenic domains that are ideal targets for a species-specific PCR assay. We have designed oligonucleotides that are highly efficient at amplifying *P. marinus* mtDNA in extracts of oyster tissues. We can detect 10 pg of *P. marinus* total DNA per ug of oyster DNA in an extract with EtBr staining of agarose gels, and 100 fg with Southern-Blot autoradiography. Additionally, a 3.2 Kbp *P. marinus* mtDNA clone contains the 5S and 16S rRNA genes as well as a ribosomal spacer domain. We have produced phylogenetic reconstructions for both rRNA genes using PAUP to evaluate lineage significance. These analyses are in agreement with other recent studies that suggest a closer affinity between *P. marinus* and dinoflagellates than between *P. marinus* and other apicomplexans. Functionally, almost all apicomplexans are strict intracellular parasites while dinoflagellates can be free-living or symbiotic with intra- or extracellular modes of parasitism. This suggests that these two classes may have been derived from a common marine 'opportunistic' protozoan parasite, with the apicomplexans diverging as they became more specialized for intracellular parasitism. *P. marinus* is not a strict intracellular parasite and therefore may retain life history characteristics more closely related to dinoflagellates. (Supported by Cooperative Agreement #NA47FL-0163 NOAA/NMFS to G.R.V.)

B9-110 THE EFFECT OF SHORT-TERM AND LONG-TERM EXPOSURE TO TURBULENCE ON THE FERTILIZATION AND EARLY DEVELOPMENT OF THE PURPLE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*. Kristina S. Mead, Hopkins Marine Station, Dept. of Biological Sciences, Stanford University, Pacific Grove, CA 93950.

Purple sea urchins living on exposed rocky coasts experience intense turbulence caused by breaking waves. Since purple sea urchins reproduce via external fertilization, released gametes are also exposed to intense turbulence. While low levels of turbulence increase mixing and therefore enhance fertilization success, excessive turbulence decreases fertilization success both because the energetic water motion rapidly dilutes the gametes, and because the small-scale hydrodynamics hinder contact between the egg and sperm.

In order to study this latter effect, gametes from *S. purpuratus* were exposed to environmentally relevant energy dissipation rates (a measure of turbulence) in a Couette cell, an apparatus consisting of two concentric, rotating cylinders. Experiments show that when eggs are exposed to sperm while in the Couette cell, only a small percent of the eggs are fertilized. Furthermore, only a fraction of the fertilized eggs develop into normal blastulae. In contrast, eggs or sperm exposed to the same energy dissipation rate on their own, prior to fertilization, do not suffer a significant loss in fertilizability, and develop into normal blastulae. This suggests that decreases in egg-sperm contact and binding are responsible for the observed decrease in fertilization success, and that changes in the egg surface structure at fertilization render the egg more susceptible to turbulence-induced damage, resulting in decreased developmental success.

While the Couette cell experiments depict the role of the short-term exposure to turbulence in the rocky intertidal zone, experiments in a shaking grid tank, in which energy dissipation rates are generated by a grid shedding vortices as it moves through water, examine the effects of the long-term exposure to turbulence experienced by gametes and embryos as they move out from the rocky intertidal zone into the mixed-layer.

B9-111 DISPERSAL OF HOLOTHURIAN LARVAE DETERMINED WITH GENETIC PROBES, Dorothy E. Medeiros-Bergen, Richard R. Olson, Janet Conroy, and Thomas D. Kocher, Department of Zoology and Center for Marine Biology, University of New Hampshire, Durham, NH 03824

Identification of marine invertebrate larvae to the species level is difficult due to morphological similarity and phenotypic plasticity. In order to study dispersal of morphologically-indistinguishable holothurian larvae in the Western Gulf of Maine, we developed a series of radioactive oligonucleotide probes for the 16S rRNA portion of the mitochondrial DNA genome. Using this technique we analyzed more than 1600 larvae over the planktonic season. Of the three species present in the plankton, *Cucumaria frondosa* larvae dominate the samples, often comprising more than 90% of the larval pool and 95% of the new recruits. Temporal differences in the planktonic distribution suggest the southward transport of *C. frondosa* into areas where adult survival is extremely low. The vertical distribution of larvae over time suggests that larvae are primarily located in the upper coastal waters. The ecological implications of the coastal transport of holothurian larvae are discussed.

B9-112 **MOLECULAR POPULATION GENETICS OF THE CALIFORNIA SEA HARE (*Aplysia californica*) USING SINGLE COPY NUCLEAR DNA MARKERS,** Mónica Medina and Patrick J. Walsh, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway., Miami, FL 33149

Aplysia californica is a large herbivorous opistobranch mollusc, endemic to the coast of California and the Sea of Cortez. It has a one year life cycle, with a planktonic larvae of approximately 35 days. *A. californica* is used extensively in biomedical research as a model organism in neurological studies. In the present study, single-copy nuclear DNA markers are being used to study the genetic population structure of *A. californica*. The results from this study should be of importance to the management of hatchery populations, and provide genetic background for neurological research laboratories.

A partial DNA library was constructed with total *Aplysia* DNA, low copy clones were sequenced to design specific primers for these regions, and PCR products were screened for polymorphic sites with restriction enzymes. At present two polymorphic loci have been detected and screened for four populations. Locus MP9303#1 (~600 bp) exhibited two polymorphic enzymes (RsaI and Sau96I) with four polymorphic restriction sites. Locus MP9303#44 (~1600 bp) exhibited four polymorphic enzymes (DdeI, HaeIII, MboI and NlaIII) with 16 polymorphic sites. Each locus presented five and eight alleles respectively. The low mean F_{ST} value (0.012) and cluster analyses show no evidence of genetic population differentiation. Genetic relationships do not seem to be based on geographic distribution and the levels of genetic distance are extremely low which is concordant with no population structure. This observation can be related to the long pelagic larval life of this species, but in order to obtain a more accurate representation of the population genetics of this organism, additional loci and populations are being surveyed.

B9-113 RECOMBINANT EXPRESSION AND PURIFICATION OF THE ANTIMICROBIAL PEPTIDE POLYPHEMUSIN FROM THE HORSESHOE CRAB *LIMULUS POLYPHEMUS*. James C. Pierce, Department of Natural Science and Mathematics, The Richard Stockton College of New Jersey, Pomona, NJ 08240

Polyphemusin is a broad spectrum antimicrobial peptide isolated from the hemocytes of the North American horseshoe crab, *Limulus polyphemus*. The physiologic mechanism of antimicrobial activity is thought to be mediated by selective disruption of cell membranes in target organisms. To date the source of polyphemusin has been purification from natural materials or chemical synthesis. I report here the recombinant expression in *Escherichia coli* and subsequent purification of a polyphemusin analog (rLim1). This analog is the natural precursor to the amidated native polyphemusin peptide. To prevent toxicity of the antimicrobial peptide in the highly susceptible *E. coli* host, a carboxyl terminal fusion protein cloning strategy provided by the maltose-binding protein gene fusion system was used. In this system the MalE-rLim1 fusion protein was up to 20% of the total cell protein and was present in the cytoplasmic soluble fraction. Release of the rLim1 peptide was accomplished by cyanogen bromide cleavage. Correct folding and sulfhydryl bond formation occurred spontaneously during peptide purification. Antimicrobial activity of recombinant polyphemusin was similar to that seen with native peptide. The possibility of generating transgenic organisms (eg. oyster) with novel immune functions using a recombinant polyphemusin vector is now feasible.

B9-114 HYBRIDIZATION AS AN EVOLUTIONARY FORCE IN MASS-SPAWNING SCLERACTINIAN CORALS, Robert H. Richmond, Marine Laboratory, University of Guam, UOG Station, Mangilao, Guam 96923

The majority of scleractinian corals studied to date are simultaneous hermaphrodites that participate in multi-species mass spawning events. Typically, combined egg-sperm clusters are released composed of between 9 - 185 eggs surrounding or embedded within a central sperm packet. These clusters usually remain intact for periods of 15 - 40 minutes prior to disruption and the first observed fertilizations. Experiments performed on acroporid corals have demonstrated that hybridization can occur between species, with viable and competent planula larvae developing as a result. Reciprocal fertilization experiments between Acropora digitifera and A. gemmifera found hybridization to be unilateral, with rates as high as 100%. Other species of Acropora were also found to hybridize, with fertilization rates ranging from ca. 30% to 60%. The specificity of coral egg-sperm interactions was demonstrated by observed barriers to self-fertilization in some species, however, such barriers were found to be time-dependent with observed rates of selfing increasing after 6 hours. While numerous species of corals were observed to spawn over a period of days, there were consistent temporal patterns in the timing of gamete release with respect to both time of night and number of days following the summer full moon. These timing differences may be interpreted as temporal, "pre-mating" mechanisms for reproductive isolation considering the dilution factor affecting gametes. Data on reproductive mode (spawning vs brooding) and species diversity patterns support the hypothesis that hybridization is a mechanism supporting speciation in mass-spawning corals.

B9-115 ORGANIZATION AND EVOLUTION OF THE OPSIN GENE IN OCTOPUS BIMACULOIDES, John W. Roberts, Kathleen Szick, Paul Nishita, and Laura Robles, Department of Biology, California State University, Dominguez Hills, Carson, CA 90747

Opsin, the apoprotein of rhodopsin, consists, in mammals, of four cytoplasmic and four intradiscal domains separated by seven helical transmembrane segments (I-VII). Bovine and human opsin genes are interrupted by four introns: intron 1, within the region encoding transmembrane helix III; intron 2, near the region encoding the carboxy-terminal end of helix IV; intron 3, near the region encoding the carboxy-terminal end of helix V; and intron 4, near the region encoding the carboxy-terminal end of helix VII.

We have used PCR amplification and nucleotide sequence analysis to determine the organization of the opsin gene in Octopus bimaculoides genomic DNA. PCR primers were based on the opsin cDNA sequence for Paroctopus delfleini (Ovchinnikov et al., [1988], FEBS Letters 232, 69-72). As expected, the exons of the Octopus gene are very similar to homologous segments of the Paroctopus cDNA, and most nucleotide differences represent silent substitutions. Consistent with the data of Ovchinnikov et al., we find a number of functionally significant amino acids in similar locations in the opsin proteins of both octopuses and the mammals.

The overall organization of the Octopus opsin gene, however, is quite different from the mammalian gene--intron 2, intron 3, and intron 4 are not present in the Octopus opsin gene. This suggests three deletion events in the evolutionary lineage leading to Octopus, since introns are present in corresponding locations in ninaE, the gene encoding the major Drosophila opsin. Analysis of opsin gene organization in bivalve and gastropod molluscs is in progress.

B9-116 BIOGEOGRAPHY, GENETIC POPULATION STRUCTURE AND EVOLUTION OF *MEMBRANIPORA* (BRYOZOA). Heidi R. Schwaninger, Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, New York 14853.
Dispersal and gene flow are poorly understood in widely dispersing marine taxa. To understand dispersal and gene flow in these systems it is important to investigate them in both the extensive geographic scale of the oceans and in an evolutionary context. I am working on elucidating the global genetic population structure of the potentially widely-dispersing cosmopolitan bryozoan *Membranipora membranacea* to test hypotheses about spatial structure and gene flow in the apparently continuous environment of the ocean. A pilot study of allozyme diversity in *M. membranacea* found little genetic differentiation among populations on the American west coast or populations in the Atlantic ocean. However, the interocean F_{st} (based on 5 polymorphic loci in Hardy-Weinberg equilibrium) was .508 and Nei's genetic distance .874, indicating large differentiation of these gene pools. Similarly, preliminary sequence data of a 455 base pair section of the mitochondrial gene coding for the large ribosomal subunit (16s) indicate 4% sequence divergence between populations of the two oceans. This study will be expanded by exploring additional mitochondrial markers, by generating mtDNA data of *M. membranacea* populations from both hemispheres and many species in the genus *Membranipora* to test hypotheses about gene flow and speciation in this potentially widely dispersing group.

B9-117 ECOLOGICAL ROLES FOR SECONDARY METABOLITES IN TROPICAL PACIFIC SOFT CORALS AND THEIR EGGS, Marc Slattery, Valerie J. Paul, Kathryn L. Van Alstyne, and Chad R. Wylie, University of Guam, Marine Laboratory, Mangiloa, Guam 96923.
The soft corals *Sinularia* spp., *Sarcophyton* spp., and *Lobophyton* spp. are common and often dominant members of the shallow reef communities surrounding Guam. As in many octocorals, these species contain a rich assemblage of secondary metabolites which appear to serve defensive roles in the adult coral colonies. *Sinularia maxima*, *S. polydatyla*, and *S.* sp. contain compounds which deter a natural assemblage of predatory fish at extract concentrations similiar to that found in the adult colonies. Bioactive compounds, typically cembranoid diterpenes, include species-specific secondary metabolites which are preferentially concentrated in the polyp-bearing tips of the colonies. In addition, a major diterpene isolated from *Sarcophyton* sp. deters feeding in an omnivorous pufferfish *Canthigaster solandri*. The same compound is found in the eggs at high concentrations implying a feeding deterrent function in the vulnerable larval stage. Intra-colony, seasonal, and ontogenetic variations in the presence of certain soft coral metabolites indicates some of these compounds may play an important role in reproductive processes. Specifically, the evolutionary significance of these compounds may be as sperm attractants or maturation hormones rather than as chemical defenses.

B9-118 GENETIC DIVERGENCE AND SPECIATION IN AN EXTREMELY YOUNG PUPFISH SPECIES FLOCK IN MEXICO FORMED BY THE GENUS CYPRINODON (CYPRINODONTIDAE, TELEOSTEI), Ulrike Strecker[1,2], Christian G. Meyer[2], Christian Sturmbauer[3] and Horst Wilkens[2]
[1] Zoological Institute and Zoological Museum, University of Hamburg, 20146 Hamburg, Germany; [2] Bernhard Nocht Insitute for Tropical Medicine, Department of Molecular Biology, Hamburg, Germany;
[3] Institute of Zoology, University of Innsbruck, 6020 Innsbruck, Austria.

The Laguna Chichancanab contains one of the evolutionarily youngest species flocks in the world, comprised of five endemic species of the genus Cyprinodon. The lake probably arose by elevation processes in which a former coastline of the Caribbean Sea was transformed into a lake. C. artifrons, the presumed sister group of the endemics still exists in coastal habitats. Sequences of the mitochondrial control region (up to 463-bp) were obtained for C. artifrons (17 individuals sequenced), and for all five endemic species (32 individuals sequenced). While nine different haplotypes were found in C. artifrons, only a single haplotype was shared with the endemics. Four additional haplotypes were found among the endemics that were most closely related to the single haplotype shared with the sister group. The deprivation in haplotypes among the endemics points to strong bottlenecking, in agreement with geological data suggesting an almost complete desiccation of the lake about 8,000 years ago. While four of the five species shared haplotypes with each other, only a single and unique haplotype was found in C. maya (12 individuals sequenced). A comparison of sequence divergences with those found among African cichlid fishes endemic to East African lakes, given equal rates of substitutions for the control region, suggests that the flock may have evolved after the desiccation event 8,000 years before present. The distribution pattern of overlapping haplotypes in four of the five species points to incomplete lineage sorting, or to hybridisation between those species. Complete reproductive isolation is only assumed for C. maya.

B9-119 LOSS OF MITOCHONDRIAL DNA DIVERSITY IN HARPACTICOID COPEPODS DUE TO CONTAMINATION SUGGESTS SUBLETHAL IMPACTS, Greg T. Street, and Paul A. Montagna, University of Texas at Austin, Marine Science Institute, Port Aransas, TX 78373
Chronic environmental impacts may accumulate in areas that experience long-term development of offshore oil and gas production. However, there is no currently accepted methodology to document, yet alone quantify, sublethal stresses of this kind. We hypothesize that the first response of populations exposed to stress is a loss of genetic diversity. This loss could lead to other impacts, e.g., loss of fitness or population decline. Harpacticoid copepods are excellent models to test this hypothesis, because this dioecious group undergoes direct benthic development and have very short generation times. Five species of harpacticoid copepods were sampled from three offshore gas platforms to determine whether animals living in heavily contaminated environments experienced a loss of genetic variability relative to less contaminated environments. The polymerase chain reaction was used to generate enough genetic material from a single copepod to perform restriction fragment length polymorphisms at the mitochondrial 16S rRNA locus. Populations of all five species were composed of a single, dominant haplotype and numerous unique haplotypes. However, the relative frequency of the dominant haplotype was significantly greater for populations located near a platform (< 50 m) than those located away from a platform (> 2 km). Low genetic variability at this locus correlated with high concentrations of sediment-bound trace metals. Harpacticoid copepod population abundance and species diversity also declined near platforms. The loss of genetic and community diversity suggests that offshore drilling may account for sublethal, long-term changes associated with environmental disturbance.

B9-120 CROSS-FERTILIZATION TESTS BETWEEN SIBLING SPECIES OF AN ATLANTIC REEF CORAL, Alina M. Szmant, Ernesto Weil, D. Elizabeth Jones, and Margaret Miller, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Cswy., Miami FL 33129.

Morphological variation in *Montastrea 'annularis'*, an important Atlantic reef-building coral, in the past has been attributed to growth responses to environmental variables, especially light. It is now proposed that this species is a complex of at least three sibling species (M. annularis = MA; M. faveolata = MF; M. franksii = MR) (Weil & Knowlton 1994: Bull.Mar.Sci. 55:151-175), which have somewhat different depth distributions, but co-occur on most reefs. While characteristic colonies of each are easy to identify, the presence of intermediate morphologies suggest hybridization. As all three spawn within hours of each other on the same 1-3 nights per year, there is no spatio-temporal barrier to hybridization. Cross-fertilization trials between these species were conducted during the August '94 spawning season to determine whether fertilization-stage barriers to hybridization exist. Trials were done twice, first with samples from 3 colonies of each species spawned in the laboratory in filtered seawater (a gamete washing procedure was used to separate eggs and sperm from each set of hermaphroditic gamete bundles), and the second time with field collected gamete bundles. Except for 2 samples, self-fertilization rates were very low. All within-species crosses resulted in planulae, as did all inter-species crosses. Larvae from each cross survived for over a week, but none settled. Within-MF crosses produced the most larvae, and MF had less hybridization success with the two other species. However, for both MA and MR, crosses between species resulted in higher fertilization and larval production than those within species. These results indicate that there are no fertilization-stage barriers to hybridization between these species. Lack of settlement success in any of our cultures precludes an assessment of hybrid larvae viability. The commonness of intermediate colony morphologies suggests, however, that significant gene flow may be occurring between species, and/or that there may be more than three species in the complex. Molecular studies will be needed to answer this question and are presently in progress.

B9-121 MOLECULAR ADAPTATIONS OF SELF/NON-SELF RECOGNITION IN EXTREME ENVIRONMENTS: C-TYPE LECTINS OF THE ANTARCTIC TUNICATE *CNEMIDOCARPA VERRUCOSA*. Gerardo R. Vasta and Adam G. Marsh, Center of Marine Biotechnology, Univ. of Maryland Biotechnology Institute, Baltimore, MD 21202.

C-type lectins are considered to play a key role in the process of self/non-self recognition in invertebrates. C-type lectins can be either soluble or membrane bound and have a calcium ion requirement for sugar binding activity. Different C-type lectins recognize a wide variety of mono- and disaccharide structures, and it is through these carbohydrate binding specificities that C-type lectins are able to recognize 'self' and 'non-self' materials. We are interested in assessing the functional roles of C-type lectins in an Antarctic tunicate to determine if extreme low environmental temperatures (-2 to 0 ^{o}C) have altered the physiological activities of these molecules. Low temperatures may alter lectin binding kinetics and ligand specificities and thus subject the C-type lectins of *C. verrucosa* to a different set of selective pressures than has been found in tropical and temperate tunicates. A serological survey of *C. verrucosa* hemolymph and tissue extracts prepared from ovaries, body wall, and epicardial lobes revealed lectin activity as evidenced through high hemagglutination titers. Cross adsorption studies suggest the presence of multiple lectins in the tissues screened. We have partially characterized two separate binding activities through direct sugar-inhibition studies. Agglutination of horse RBCs was strongly inhibited by GlcNAc and fetuin, while agglutination of bovine RBCs was strongly inhibited by bovine submaxillary mucin and lactose. We are currently conducting the molecular characterization of these proteins and analyzing their fine carbohydrate specificities. (Supported by NSF Grant MCB-91-05875 to G.R.V.)

B9-122 High gene flow among populations of the starfish, Linckia laevigata, over the West Pacific and Indo-Malay region. Suzanne T. Williams and John A. H. Benzie, Australian Institute of Marine Science, PMB 3, Townsville QLD 4810, Australia

Genetic variation in 21 populations of the common coral reef starfish, Linckia laevigata, showed no significant population differentiation over distances of greater than 7000 km, and high levels of diversity. The lack of genetic structuring implies high levels of gene flow, and therefore sufficient dispersal of L. laevigata larvae to prevent genetic differentiation of populations, throughout the West Pacific Ocean and Indo-Malay region. This survey confirms the occurrence of a marine species with a widespread geographic range with effective high gene flow throughout a large population. Recent genetic surveys of several marine species have indicated that species, with apparently large ranges, actually consist of several unrecognized sibling species. Temporary isolation of parts of the present large species range would provide a mechanism for increasing overall diversity through population differentiation. Variants would be transmitted by larval dispersal throughout the entire species range after the removal of barriers. High levels of polymorphism could be maintained in the large population through rapid (in geological time) and recurring disturbances to gene flow.

B9-123 NATURAL HYBRIDIZATION WITHIN THE CORAL GENUS *ACROPORA*?, Bette L. Willis[1], Carden C. Wallace[2] and David J. Ayre[3], [1]Department of Marine Biology, James Cook University, Townsville, Q 4811, Australia, [2]Museum of Tropical Queensland, Townsville, Q 4810, Australia, [3]Department of Biology, University of Wollongong, Wollongong, NSW 2500, Australia

Synchronous spawning of many congeneric species of coral on reefs throughout the Indo-Pacific raises the possibility that hybridization is part of the fertilization ecology of reef corals. We have found that many morphologically distinct species of *Acropora* readily hybridize *in vitro*, and that the larvae develop and settle normally. Comparisons of the survival and growth of juveniles resulting from within-species and hybrid crosses indicate that there are no apparent pre- or post-mating isolating mechanisms mitigating against the occurrence of natural hybrids on reefs, although it is not yet known whether such hybrids are sexually viable. Between 300 and 1500 juveniles from hybrid crosses involving *A. pulchra*, *A. millepora*, *A. cytherea* and *A. formosa* have been followed for up to 2 years. At one year, *A. millepora-formosa* and *A. millepora-pulchra* juveniles had survived in equivalent numbers and attained a greater mean size than juveniles from most *A. pulchra* and *A. formosa* within-species crosses. Electrophoretic surveys of populations of *A. millepora* and *A. pulchra* in the central Great Barrier Reef region suggest that gene flow *is* occurring between these two species. Genetic distances (Nei's) between 8 populations of *A. millepora* and 6 populations of *A. pulchra* were less than 0.15 ($n > 45$ corals per population, populations separated by up to 80k). Three populations of *A. pulchra* were genetically more similar to populations of *A. millepora* ($D < 0.01$) than to other populations of *A. pulchra*. Evidence of interbreeding and gene flow between species suggests that reticulate evolution occurs within the Scleractinia and has profound implications for studies of the population genetics, phylogenetics and evolutionary biology of broadcast-spawning corals.

Late Abstracts

SEQUENCE VARIATION IN MITOCHONDRIAL 16S rRNA OF THREE SUBCLASSES OF ANTHOZOANS (PHYLUM CNIDARIA), J. Ewann Agenbroad*, Scott C. France**, Lauren S. Mullineaux*, *Department of Biological Oceanography, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, **Department of Zoology, University of New Hampshire, Durham, NH 03824

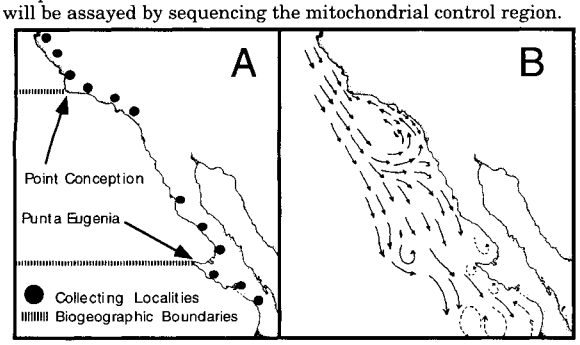
In an initial survey of genetic variation for a study of dispersal among benthic fauna of deep-seamounts, we have sequenced a portion of the mitochondrial 16S rRNA gene in 26 species from 3 subclasses of Anthozoa. Our data has revealed length variation of 40 % between subclasses, with fragment lengths from approximately 545 to 911 bp. Included in this survey were 5 hexacoral (3 actinarians, 2 scleractinians), 16 octocoral and 3 antipatharian species. The 5' and the 3' ends of the sequence are relatively conserved across all taxa, but the center region (350 bp in the longest haplotype) tends to be highly divergent between subclasses. We found no sequence variation within any species for which multiple individuals were sampled. The 16S fragments of the antipatharians (Subclass Ceriantipatharia) were roughly 300 base pairs longer than those of any other taxa. The Ceriantipatharia is a small subclass with 2 orders (Antipatharia, Ceriantharia) which were formerly classified within the Hexacorallia. To examine the taxonomic breadth of the large INDEL, we sequenced a cerianthid anemone from the shallow waters of Florida. The length of the 16S fragment more closely resembled the octocorals than the antipatharians. A parsimony tree built using the 3' end of this fragment places the cerianthid anemone outside of a clade containing both the Antipatharia and the actinarians. This suggests the large INDEL is a recent development solely in the order Antipatharia. The remaining octocorals cluster together as expected from traditional, morphologically-based taxonomy.

REPEATED SEQUENCES IN THE GENOME OF *PENAEUS VANNAMEI* AND OTHER DECAPODS. Joseph C. Bagshaw, Dept. of Biology and Biotechnology, Worcester Polytechnic Institute,Worcester, MA 01609

Sequences repeated in the genome, especially those that are widely dispersed, can be useful as molecular markers for a variety of inter- and intrapopulation comparisons. We have cloned to date 67 Sau 3AI fragments containing sequences repeated in the genome of the Pacific white shrimp, *Penaeus vannamei*. Of 11 clones tested as hybridization probes against Southern blots of genomic DNA fragments, nine were found to be highly abundant and widely dispersed. This group included three dinucleotide and one trinucleotide microsatellites. The remaining two, plus several additional clones, represent a tandemly repeated sequence of 139-188 bp. The variability is due to the presence of 6 - 15 tandem copies of a pentanucleotide embedded within the larger tandemly repeated unit. We refer to this structure as PVS 1. Using synthetic oligonucleotides based on repeated sequences and a primer extension strategy, we can observe restriction site polymorphism between individuals within and among populations, including selectively bred families. PVS 1 cross-hybridizes strongly with discrete restriction fragments of DNA from crayfish and lobster. Surprisingly, PVS 1 cross-hybridizes only weakly with DNA from *Peneaus monodon*. We have also cloned ribosomal RNA genes and histone genes from *P. vannamei*. The rRNA genes are polymorphic within and between populations. The polymorphism appears to result primarily from a series of discrete length differences in the intragenic spacer region.

WHAT ARE THE FORCES MAINTAINING BIOGEOGRAPHIC BOUNDARIES? A POPULATION GENETIC PERSPECTIVE. Cunningham, Cliff. Duke Zoology.

Knowing why species ranges end where they do is essential to understanding how species will respond to changes in global climate, and to understanding why ranges have changed in the past. We are investigating the hypothesis that reduced larval dispersal due to ocean currents -- not thermal gradients -- maintain a pattern of abrupt change in species compositon across Pt. Conception in California and Pta. Eugenia in Baja California. The figure below show predominately North-South currents across both points, which may explain why these headlands represent the *northern* boundaries for many more species with planktonic larvae than they do *southern* boundaries. To ask how ocean currents affect larval migration we have initiated a detailed molecular population genetic study of the barnacle *Balanus glandula* and *Tetraclita rubescens,* which are common across Pt. Conception and Pta. Eugenia. We predict that if South-North migration is impeded by ocean currents, migration across the boundaries will be depressed, and unique alleles will tend to accumulate to the South. Variation will be assayed by sequencing the mitochondrial control region.



RESOURCE PARTITIONING AND THE RADIATION OF SYMBIOTIC CORAL-REEF SHRIMP, J. EMMETT DUFFY, SCHOOL OF MARINE SCIENCE, COLLEGE OF WILLIAM AND MARY, GLOUCESTER POINT, VA 23062-1346.

Sponge-dwelling snapping shrimp (*Synalpheus*) comprise one of the most species-rich genera of marine crustaceans, with >100 described, and many undescribed, species, 20 or more of which can be found living sympatrically. The narrow host ranges of these shrimp, together with pronounced segregation of closely related species into different hosts, suggest that adaptation to new host sponges, and consequent speciation, represents an important driver of cladogenesis in this genus. At the population level, this hypothesis is supported by genetic (allozyme), demographic, and behavioral divergence between shrimp demes occupying different host species. But what is the role of geographic isolation in this process? To find out, I mapped host use and geographic distribution onto a cladogram of four cryptic species in the "*S. brooksi*" complex, based on a ~600-bp sequence of mt COI and ten allozyme loci. The three most closely related shrimp species were partially to completely allopatric, and overlapped in host range, indicating that speciation must have been initiated by spatial isolation rather than by host shifts. Despite overlap, however, no two of these species used the same suite of hosts. Moreover, analysis of another complex of apparently older species showed no overlap in host use at all. These data suggest that the pronounced "resource (host) partitioning" seen in sponge-dwelling shrimp may result from competitive character displacement between recently diverged sister species. Ultimately, the high diversity of *Synalpheus* appears attributable to a combination of extraordinarily restricted gene flow and divergent selection imposed by specialization on different resources.

DEPTH-RELATED SEQUENCE VARIATION IN DEEP-SEA AMPHIPOD POPULATIONS FROM THE NORTH ATLANTIC AND NORTH PACIFIC OCEANS, Scott C. France and Thomas D. Kocher, Department of Zoology, University of New Hampshire, Durham, NH 03824

The cosmopolitan, deep-sea scavenging lysianassoid amphipod *Eurythenes gryllus* has been trapped in both northern and southern hemispheres and polar regions of all oceans, at depths ranging from 1000 - 6500 meters. There have been conflicting views in the past over whether worldwide populations of *E. gryllus* are conspecific. Although new species have been proposed, at present all variants belong to the species "*gryllus*." There is only one other species in the genus, the pelagic *E. obesus*.

Using DNA sequence data from the mitochondrial 16s rRNA gene, we have begun to address the variation among populations of *E. gryllus* from the north Atlantic and Pacific Oceans. Populations have been sampled from 4 sites in the Eastern North Atlantic (ENA) ranging in depth from 2900 - 5100 meters, 1 from the Western North Atlantic at 1300 m (WNA), and 1 from the central North Pacific at 5700 m (CNP). Our initial results indicate sequence similarity correlated with depth of populations. All abyssal (>4500 m) ENA sites shared identical haplotypes, and only a single transition was found between bathyal sites (<4000 m) on opposite sides of the Atlantic ocean basin. Moderate levels of variation between abyssal populations from different oceans (CNP vs. ENA - 1.7% sequence difference) contrasted with higher levels of variation between bathyal and abyssal populations, even within the eastern North Atlantic (3.9 % seq. diff.). Little or no sequence variation was found among individuals within a population (max. 0.2 % seq. diff.). The low level of differentiation between abyssal populations living in different ocean basins contrast with the relatively high levels associated with bathyal vs. abyssal comparisons, and suggest that *E. gryllus* maintains a widespread horizontal distribution but a small vertical distribution. The level of divergence between abyssal and bathyal populations suggests that separate species status may be appropriate.

ANIMAL PHYLOGENY: RIBOSOMAL RNA STUDIES OF ASCHELMINTHES, James R. Garey, Laura Y. Mackey, Jacqueline M. Brooks, Birgitta Winnepenninckx, and Thierry Backeljau, Department of Biological Sciences, Duquesne University, Pittsburgh PA 15282 USA; Departement Biochemie, Universiteit Antwerpen (UIA), Universiteitsplein 1, B-2610 Antwerpen, Belgium; Koninklijk Belgisch Instituut voor Natuurwetenschappen, Afdeling Malacologie, Vautierstraat 29, B-1040 Brussel, Belgium.

The Aschelminthes is a loose collection of at least 8 phyla that have historically been grouped together because the lack an eucoelom has been interpreted as a pseudocoelom. The phylogenetic relationships of these animals to one another and to the rest of the animal kingdom are controversial and uncertain. We are re-examining these relationships using the DNA sequence of the entire 18S ribosomal RNA gene. New sequences from a representative of the aschelminth phyla Rotifera, Priapulida, Gastrotricha, Nematomorpha and previously published sequences from Nematoda and Acanthocephala have been analyzed with sequences from representatives of a variety of other animal phyla. The sequences were aligned and trees prepared by the Neighbor-Joining and Maximum Parsimony methods. The results indicate that Priapulida are clearly protostomes, Acanthocephala and Rotifera form a clade that may be a sister group to the protostomes, and Nematoda appear to be the basal members of the Eumetazoa and may form a clade with the Nematomorpha, while the position of Gastrotricha is uncertain. This analysis is consistent with recent ultrastructural evidence which suggests that the ancestral bilateral animal possessed an eucoelom and that the pseudocoelom results from the modification of the eucoelom. The Aschelminthes are clearly a polyphyletic assemblage of animal phyla and it is likely that the pseudocoelom arose several times during animal evolution by the modification of the ancestral eucoelom.

MOLECULAR PHYLOGENETIC RELATIONSHIPS OF LUMINESCENT CYPRIDINID OSTRACODE CRUSTACEANS: PATTERNS OF EVOLUTION OF BIOLUMINESCENCE AND SIGNALING BEHAVIOR, Elizabeth Torres, James G. Morin, and Anne C. Cohen, Department of Biology, University of California, Los Angeles, Los Angeles, CA 90024-1606.

Males of many species of luminescent cypridinid ostracodes (7 genera containing over 60 species) from the Caribbean utilize species-specific light signals for courtship. Relationships among these species, and their relationship to non-signaling and non-luminescent members of the family have been reconstructed on the basis of morphology. Cohen and Morin proposed that the signaling ostracodes belong to two separate monophyletic clades within the family. One class of general display patterns occurs within each of the seven genera while others are restricted to certain species, and thus appear to be derived. In order to further resolve the relationships among signaling genera, luminescent genera lacking signaling behavior, and non-luminescent genera we have sequenced a portion of the 16S ribosomal mitochondrial gene from representatives of the Cypridinidae. The resulting molecular phylogeny is used as an independent test of the morphologically based relationships, and serves to test the monophyly of luminescent signaling behavior in the family as well as clarify relationships among all luminescent taxa. Additionally, we have sequenced a number of species within a diverse signaling genus (Photeros) in order to investigate the evolution of signaling patterns between more closely related taxa.

ENGRAILED SEQUENCE AND EXPRESSION IN THE MOLLUSCA: A DEVELOPMENTAL AND PHYLOGENETIC SYNTHESIS, Charles G. Wray and David K. Jacobs, Department of Biology, University of California, 621 Circle Drive South, Los Angeles, CA 90024-1606

The *engrailed* gene, which includes a homeodomain, is highly conserved throughout the metazoa. This segment-polarity gene is used to investigate the homology of developmental patterns both across the diverse molluscan classes and relative to the protostome ancestors of the molluscs. The expression of *engrailed* demarcates segmental fields in annelids and arthropods. Expression studies and phylogenetic reconstructions suggest that metameric units in annelids, arthropods and molluscs are developmentally homologous. Phylogenetic hypotheses are based on nuclear small subunit rDNA, engrailed DNA, and a large morphologic data set analyzed within a total evidence framework. *engrailed* DNA sequence is presented for the five major molluscan classes. Expression patterns of engrailed in several molluscs suggests that 1) serial repetition of organs and muscles is primitive and 2) the conchiferan classes have lost serial patterning in their bodyplans.