C-DEBI Evolution Theme Team Meeting

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The George and MaryLou Boone Center for Science and Environmental Leadership,
Wrigley Marine Science Center,
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PARTICIPANTS

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PURPOSE

C-DEBI is a NSF Science and Technology Center funded with $25M for five years (with a possible renewal for another 5 years), and dedicated to the study of the biosphere that exists at and beneath the ocean floor. Studies (Gold, Whitman) suggest that despite the low apparent biomass in these environments, the large areas covered result in the deep biosphere harboring the majority of biomass on the planet. Recent projects have begun to delineate the abundance and diversity of organisms present in the subseafloor biosphere and their global geochemical importance. With the establishment of the Integrated Ocean Discovery Program in 2003, sampling and *in situ* experiments for sub-seafloor biological studies have been on the rise. C-DEBI has three specific aims: 1) to coordinate, integrate, support and extend the science associated with IODP drilling projects; 2) to foster and educate an interdisciplinary community of researchers in deep sub-seafloor biosphere research; and 3) to educate, inform, and translate knowledge of the deep sub-seafloor biosphere. To facilitate these aims, C-DEBI provides funding in the form of graduate and post-doctoral fellowships, a research and travel exchange program, and direct research support.

C-DEBI has four major themes on which it is focusing its research efforts: 1) biological and biogeochemical activity in the deep sub-seafloor biosphere; 2) extent of biogeographical distribution of life on earth; 3) environmental and nutritional limits of life; and 4) evolution, adaptation and survival. These themes are developed and brought from the conceptual to the practical by Theme Teams. The Theme Teams, organized by the Theme Team Leader, are composed of both experts and novices from both within and without the established deep-sea research community to bring both long-term knowledge and experience, and fresh perspective and enthusiasm to the process.

The purpose of this inaugural convening of the Evolution Theme Team was to establish a baseline for our understanding of the deep sub-seafloor communities and their environments, determine what questions pertaining to evolution are of most immediate concern, and determine whether and how such questions can be addressed within the current and expected future structure of the IODP and C-DEBI programs. The results of this discussion are meant to guide future research efforts and the development of new scientific drilling programs and investigations within C-DEBI. We also seek to inform the preparation of requests for proposals to ensure C-DEBI goals are met, applicants thereof so they are aware of our focus, reviewers thereof so they have a frame of reference from which to judge proposals, the broad scientific community so they understand the purpose and significance of the work, and the general public so they might understand the relevance of the research to their world (and tax dollars).

MEETING SUMMARY

Caveats

The establishment of a new research program first requires an understanding of the current state of field in question. In this case it is also important to establish the technical limitations of deep-sea science in order to understand what types of project are possible. There are still questions about contamination of samples due to the drilling process, changes that may occur in cores due to the long transit times from bottom to surface and from surface to storage. It is also unclear how the formation of a borehole affects the hydrology and biology of the system and whether a borehole is still representative of the environment. It was generally agreed that archived samples were unlikely to be appropriate for biological studies. These concerns are general to deep-sea research, but may have specific impact on studies designed to look at evolutionary questions. These concerns also strongly demonstrate the need for additional deep-sea and sub-seafloor investigation initiatives, which this community is uniquely poised to be able to address.

Another technical concern is the fact that the overhead involved in obtaining samples makes them quite precious. For example, many sediment samples have very low biomass. While it has been clearly demonstrated that there is sufficient material for molecular techniques, an added level of practice and preparation on the scientist's part is required to ensure success. We agreed that support for developing techniques in surface environments that could then be applied to deep-sea samples or *in situ* experiments would be a good use of C-DEBI funds.

Jon Eisen gave a brief overview of the GEBA (Genomic Encyclopedia of Bacteria and Archaea) Project. GEBA is a genome sequencing project funded by the DOE/JGI in which genome projects based on 1) under-representation of the phylogenetic branch in the sequence databases and 2) culturability of the organism. This project is already demonstrating how much greater diversity is at both the organismal, genetic and metabolic level than we may have previously understood. An analysis of the first 50 genomes from this project indicates that another 1000 representative genomes will need to be sequenced just to cover the diversity of cultured organisms, which is estimated to be 0.5% of total microbial diversity. It could take another 12,000 genomes to cover 1/2 the total diversity known in 2007. This level of diversity could hamper our ability to analyze genomic data generated from these environments and calls for caution in interpretation of such data.

Sediment communities

One of the topics discussed was that of life in the sediment. It is known that there are very low nutrient levels, and transport is diffusion controlled. This leads to very low biomass and very low metabolic rates. How have these organisms adapted to a low-nutrient environment? A metagenomic survey suggests organisms living in the sediment have little or no chemotactic ability. Do they survive in some sort of stasis, waiting for conditions to change so they can bloom, or are they slowly starving to death. In addition, basic questions about generation time, DNA damage and repair, and mutation rates need to be addressed. If an organism has a very slow growth rate due to a lack of energy in the system, does it evolve more slowly? Nothing is known about genetic exchange between individuals that live in apparent isolation. Are there virus or plasmid vectors working in the community? Do dead cells act as a reservoir of genetic material? There are many research avenues available for study in the sediment communities.

The crustal continuum

Evolution questions surrounding igneous ocean crustal microbial communities largely revolved around the ideas of isolation and dispersal. Craig Moyer spoke on his continuing research on Zetaproteobacteria and their distribution. Taking T-RFLP data from three different sites, he saw three clusters. Two of the zetaproteobacterial OTUs are found across the Pacific ocean (12 sites), but there are some that are endemic to specific regions. Two OTUs were exclusively found in the subsurface. While there was no significant relationship between vent chemistry or temperature and diversity, he did find a significant correlation with distance class, suggesting biogeographical isolation between these populations. Focusing on specific well-studied groups, as this study did, provides a better opportunity to look at evolution. This study also highlights the importance of sampling many sites, with varying environmental conditions in order to strengthen statistical tests. One idea to leverage this system to learn about the evolution of the Zetaproteobacteris would be to take samples from different areas and compare distance signature and evolutionary rates within various genes. Such a study would allow the teasing apart of the evolutionary signature of the species from that of the individual genes.

Jennifer Biddle spoke on exploration of previously unknown metabolisms (ethane and propane producers) and posed questions about evolution at the interfaces such as sulfate-methane transition. Chloroflexi appear to be one of the most abundant bacterial taxa in sediments with high organic carbon levels. What is the significance of this observation?

Virgin Oceanic

C-DEBI is part of the scientific advisory board for the Virgin Oceanic Five Dives project. Chris Welsh, a founder of the Five Dives project and the Chief Pilot for the sub, gave a presentation on the project, giving a history and description of the submersible, the support vessel (*Cheyenne,* a 125 foot catamaran), and the overall project. The sub holds a single person in its carbon fiber, aluminum and glass pressure module, and has payload space for scientific experiments. Payloads must be neutrally buoyant. Descending at 350ft/min, it can achieve the deepest depths in about 2 hours, which gives it about 2 hours of bottom time in which it is capable of transiting 10km. In addition to the sub, several seafloor landers have been designed to expand the experimental mission of the project. The current plan is for a maximum bottom time of 48 hours for the landers (any given mission schedule would be to deploy the landers on day 1, do sub mission the next day, recover the landers on day 3 and depart for the next site), however longer-term experiments are possible, but we would need to provide our own landers. Virgin Oceanic is very committed to scientific partnership, although it's unclear what kind of sub-seafloor science could be performed from these platforms. There is also a very short development time for experimental payloads or landers, since they wish to begin dives in 4-5 months. Anyone interested in either crewing the support boat or developing and experimental package for the sub or a lander should contact the C-DEBI office for further information.

Outreach

One of the missions of C-DEBI is to educate and inform the public of the research it supports and what it tells us about our planet. Cindy Joseph spoke on outreach activities. Using the California state standards as an example, she has developed an exercise to teach 7th grade students the concept of phylogenetic analysis based on 16S tag sequences. The addition of examples from cutting edge research on evolutionary principles makes the material fresh and more accessible for both instructors and students. C-DEBI accepts proposals focused on developing outreach/educational materials.

Summation

The goal of this meeting was to develop specific research avenues that C-DEBI should be invested in exploring. We have identified several aspects of the deep biosphere that lend themselves to evolutionary study. The first is the concept of isolation. There are many physical and chemical barriers separating sub-seafloor communities from the surface marine communities. Microbial communities in sediment are isolated from other communities and perhaps even from each other. Trench environments appear to be isolated from other environments. Craig Moyer's work on the Zetaproteobacteria shows the feasibility of detecting and studying isolation and distance effects. Comparing the evolution and ecology of a deep-sea system to that of another isolated community, could reveal the physical, chemical, and biological parameters of microbial community isolation. An examination of trends in gene content or mutational rate of particular genes could lead to the discovery of correlations between environmental parameters and evolution of particular genes or species. This, in turn could lead to the development of models for the evolutionary processes at work.

Related to the concept of isolation is that of dispersal. There appears to be overlap in the membership distant ridge and vent communities. How do organisms move from one to the other through an environment very different from the one in which they thrive? Is there endemism in bacterial communities? At Lost City, the age of the chimneys seems to be a determinant of community structure. Dispersal of genes can also be studied. Adaptation to environments in the sub-seafloor could be achieved by acquiring beneficial metabolic capabilities via lateral gene transfer through viral or plasmid vectors. There is much literature on plant and animal dispersal vs distribution models that can guide these research avenues.

The deep, dark biosphere also offers an excellent opportunity to study extremely slow growth and nutrient limitation on evolutionary rate. As mentioned above, the sediment environment is very energy-poor and metabolic rates are extremely low. What are the sources of mutation in this environment? How do the cells handle DNA damage with such a limited energy budget? When and how does repair of such damage occur? If DNA damage is occurring and not being actively repaired due to energy limitation, it could be possible to increase DNA yields and quality from preparations by adding DNA repair enzymes to the isolation process. This technique has been used by researchers studying ancient DNA to good effect.

The deep and subsurface marine environment forces organisms to use energy sources (electron donors and acceptors) and chemical environments that are unusual or unknown at the surface and thus poorly understood. Evolution of these novel metabolic pathways is of great interest. Are these systems generally adaptable to various environmental conditions or did they all originate from distinct backgrounds? Comparing and contrasting the ecological metrics of genes is a good way to decouple the evolution of the species from the evolution of the gene. By comparing diversity measures of different gene classes one can estimate rates of evolution and determine which activities have most recently arisen. A goal might be to create a model that allows the prediction of variability based on ecological parameters.

There are now a number of good model organisms from the deep biosphere that could be the focus of evolutionary studies:

- Dehalococcoides
- ζ-proteobacteria (Mariprofundus)

- ε-proteobacteria
- Sulfate-reducing δ-proteobacteria
- Planctomycetes
- Thermococcus
- Methanocaldococcus
- Miscellaneous Crenarchaeal Group (MCG) archaea

Properly designed studies are critical to any scientific endeavor. The most robust way to perform evolutionary studies is with multiple isolates from multiple locations. The physical and chemical properties of the system must be either well known, or measurable to examine mechanisms of isolation or dispersal. Determining the variability and predictability of environmental conditions will be important for correlative studies. These types of transect time course experiments are difficult within the confines of the IODP. Perhaps the best approach would be a mega-proposal (i.e., complex drilling proposal to the IODP; see www.iodp.org) which involved visiting several sites several times, although lacking that, the accumulation of data over time from various drilling sites could (should?) provide the necessary data if these questions are kept in mind during experimental design for shorter term projects.

Proselytizing and Converting

We will endeavor to grow the cadre of evolutionary biologists in the C-DEBI community by publicizing the musings of this Theme Team and targeting distribution of Fellowship and RFP announcements to existing evolutionary biology networks, such as the Society for the Study of Evolution, the National Evolution Synthesis Center (NESCENT), McMaster University's Evolution Directory (EvolDir), the National Center for Ecological Analysis and Synthesis, and our sister STC BEACON at Michigan State. We greatly enjoyed the perspective of Jonathan Eisen at the meeting, and will continue to recruit outside experts to our Theme Team meetings.

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