Meeting report

Shelling out for genomics

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A report on the symposium 'Genomic and Proteomic Approaches to Crustacean Biology' held as part of the Society for Integrative and Comparative Biology 2006 Annual Meeting, Orlando, USA, 4-8 January 2006.

Consistent with its goal of integrating diverse fields studying basic and applied problems in biology, the Society for Integrative and Comparative Biology showcased a symposium on the genomics and proteomics of crustaceans at its recent annual meeting. This symposium emphasized the future prospects for crustacean genomics by highlighting two themes - an explosion of sequencing efforts and the implementation of physiological genomics approaches in crustaceans.

First crustacean genome sequence

Crustaceans comprise a major clade of arthropods, with thousands of extant species (see the Tree of Life web project [http://tolweb.org/tree?group=Arthropoda]). They have significant economic, ecological, and scientific importance, but have received little attention from molecular biologists beyond efforts to assess their phylogenetic relationships. Although the list of available crustacean DNA sequences is growing exponentially, it still numbers less than 100,000 (Figure 1), so there has been little grist for the genomics mill. But this is about to change - and change substantially. John Colbourne (Indiana University, Bloomington, USA), representing the Daphnia Genomics Consortium, reported on the sequencing of the genome of a cladoceran, the water flea *Daphnia pulex*. The selection of *D. pulex* was based on two considerations: genome size and ecology. The vast majority of crustaceans have genomes larger than that of *Homo sapiens* (see [http://www.genomesize.com] for a comparison of sizes), which is a significant source of inertia, but the *D. pulex* genome is only around 200 Mb. Ecologically, *D. pulex* populations show intriguing physiological and anatomical responses to changes in their physical environments such as shifts in pH or introduction of toxic metals, and to changes in predator abundance or type. The assembly and initial annotation of the *D. pulex* genome is expected to be completed by early 2007.

The impact of this genome will be felt far and wide as a lodestone for the comparative genomics of crustaceans and as an important outgroup for genomics studies of other arthropods. It will also invigorate ongoing cDNA sequencing efforts reported at the symposium, all of which are from the Decapoda (lobsters, crabs and shrimp). David Durica (University of Oklahoma, Norman, USA) reported the sequencing of cDNAs from blastema tissue of fiddler crab (*Celuca pugilator*) legs during limb regeneration, thereby identifying genes potentially regulated by ecdysteroids. Thomas Shafer (University of North Carolina, Wilmington, USA) has sequenced cDNAs from epidermis of the blue crab *Callinectes sapidus*. He identified at least 45 cuticular proteins, some predicted to form the flexible arthrodial cuticle in crustacean joints, others to form the calcified cuticle. David Towle (Mt Desert Island Biological Laboratory, Salisbury Cove, USA) reported cDNA sequences from the green shore crab *Carcinus maenas* and the lobster *Homarus americanus* that have helped identify enzymes, ion exchangers and pumps involved in osmoregulation, a significant challenge faced by euryhaline species (that is, species adapted to a wide range of salinity) such as *C. maenas*. cDNAs are also being sequenced from other crustaceans with the aim of producing cDNA microarrays to study gene expression, as discussed later.

A common finding in these projects was that about 50-60% of new crustacean sequences lack a significant match in the

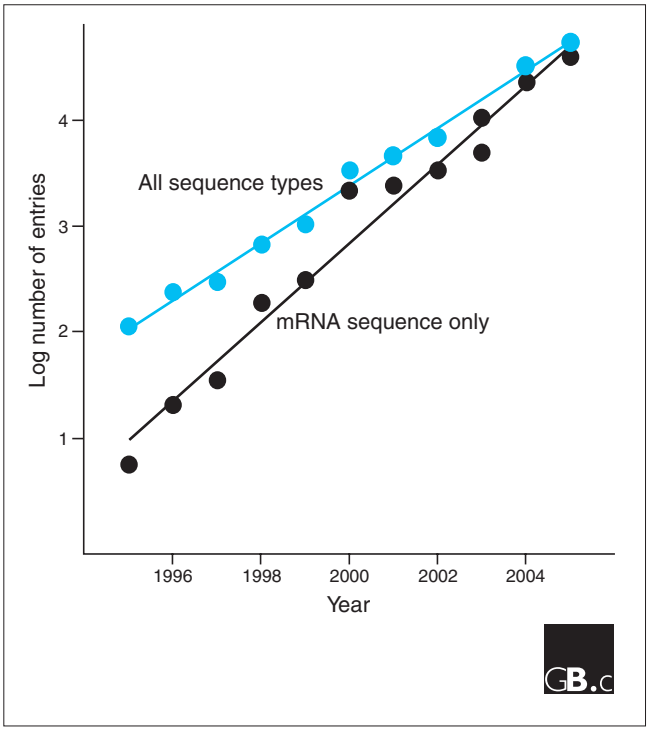

Figure 1
The exponential growth of crustacean entries in the nucleotide databases of the National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov].

public sequence databases. A major contributing factor is certainly the sequencing of ends of cDNAs, such that untranslated regions are over-represented. Another contributing factor may be the dearth of crustacean sequences combined with the large evolutionary distance of crustaceans from species whose genomes have been sequenced (the closest being insects). Plans for additional whole-genome sequencing were discussed informally, with hopes centering on the decapod with the smallest genome, *C. maenas*, and on a second *Daphnia* species.

Physiological genomics in crustaceans

Physiological ecology is the discipline currently driving crustacean genomics. *Daphnia* species are important indicators of the health of lakes and ponds, so their transcriptional responses to toxins and environmental stresses are of broad interest. Colbourne reported the analysis of cDNA libraries from populations exposed to aquatic stressors such as pH change and heavy-metal pollution as a clone-and-count method of mRNA expression profiling. The results imply that a majority of mRNAs change in abundance in response to most of these stresses - an amazing result given that expression profiling in laboratory model organisms typically detects statistically significant changes in less than 10% of the mRNAs tested. This surprising finding was supported by the report from Jonathon Stillman (San Francisco State University, California, USA) that porcelain crab (*Petrolisthes cinctipes*) cDNA microarrays reveal responses to thermal stress that are just as diverse. That two crustaceans show such broad responses raises questions as to whether environmental stresses have a greater impact in crustaceans than in other animals, whether genetic diversity is a contributing factor, and whether transcriptional regulation is especially sensitive to environmental conditions. Robert Chapman (Hollings Marine Laboratory, Charleston, USA) reported new bioinformatics approaches employing fractal mathematics to analyze microarray data on crustacean responses to environmental stress. This is an intriguing approach in which the global response and its component parts, such as clusters of related mRNAs, might both be apparent in a single integrated analysis.

Nuala O'Leary (Hollings Marine Laboratory, Charleston, USA) is using cDNA microarrays to investigate the effect of viral infections in the Pacific white shrimp (*Litopenaeus vannamei*) hepatopancreas. The transcriptional changes observed may help efforts to ameliorate the effects of infection on shrimp, which constitute 25% of all seafood consumed in the United States. O'Leary also revealed some success in using RNA interference, in the form of double-stranded RNAs to suppress individual mRNA expression, welcome evidence that this potent mechanism of viral defense is present in crustaceans. Even more sophisticated methods of studying gene expression, such as *in vivo* gene targeting and transgene insertion, need to be developed.

One of us (T.M.) reported the use of cDNA microarrays and differential amplification to investigate olfaction in *H. americanus* and the spiny lobster *Panulirus argus*. Specific markers were identified for the olfactory sensory neurons, their glial cells, reactive epithelial cells at sites of proliferation and regeneration, and secretory cells, the latter revealing a new exocrine gland in the olfactory organ, which has been named the aesthetasc tegumental gland. Nora Terwilliger (Oregon Institute of Marine Biology, Charleston, USA) reported analyses of the evolution of arthropod hemocyanins and related genes, including the prediction of hypoxia-response elements in their promoters. Many crustaceans experience fluctuations in oxygen tension, a problem that is exacerbated by human activity.

Don Mykles (Colorado State University, Fort Collins, USA) reported the first use of proteomics in a crustacean (the red land crab *Gecarcinus lateralis*). These experiments targeted the endocrinology of molting and identified numerous proteins, including proteins that bind molt-inhibiting hormone, that are candidates for regulating molting.

None of the crustacean cDNA microarrays described above is yet sufficiently broad in its coverage of the genome and sophisticated enough in its bioinformatics to provide a

systems-level view of function - that is, where activated biological processes can be identified via statistically over-represented functional groups among the affected mRNAs. Coulborne reported that coverage of the genome by the *Daphnia* microarray should soon achieve this ideal, however.

Crustaceans are an ancient and highly successful clade whose members have evolved into niches that span vitally important marine and aquatic environments. Crustacean genomics is therefore poised to have a significant impact on our understanding of environmental and physiological ecology. Even though significant technological hurdles remain, this symposium was a justifiable celebration of significant achievement and the dawning of the age of crustacean genomics.