Meeting report

High society (of nematologists)

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A report on the 43rd annual meeting of the Society of Nematologists (SON), Estes Park, USA, 7-11 August 2004.

Nematodes are ubiquitous animals; even the most barren habitats (such as the deep-sea abyssal plains) support 10^5 individuals per square meter, whereas productive habitats (such as agricultural fields) support up to 10^8 nematodes per square meter. Although most research on nematodes is focused on the free-living model *Caenorhabditis elegans* and species important as human or veterinary parasites, the Society of Nematologists (SON) largely represents scientists interested in the other species. Two speakers in the plenary session, Diana Wall (Colorado State University, Fort Collins, USA) and Byron Adams (Brigham Young University, Provo, USA), gently chided the SON membership for not embracing contemporary biology more broadly - with the question "have we taken our blinders off?" - somewhat ironically given the recent inroads genomic approaches have made towards the understanding of nematode phylogenies, evolution and function.

The tight integration of genomics with cell biology was especially evident in a symposium on plant-nematode interactions chaired by Kris Lambert (University of Illinois, Urbana, USA). Lambert's earlier work identified a chorismate mutase gene in the plant-parasitic root-knot nematode and postulated that this had been acquired from a bacterial donor via a horizontal gene transfer (HGT) event. Although the function of this enzyme in the host-parasite interaction remains speculative, Lambert's demonstration at the meeting of multiple forms of the protein does argue for a genuine role, although this awaits confirmation from an RNA interference (RNAi) experiment. Chris Taylor (Donald Danforth Plant Science Center, St. Louis, USA) described a microarray approach for profiling transcriptional changes in *Arabidopsis* membrane transport proteins that are induced by root-knot nematode invasion. Importantly, these findings support a 40-year-old hypothesis that the feeding cells elicited by the parasite are functional transfer cells (plant cells specialized for short-distance solute transport). Included in Taylor's list of plant genes responsive to root-knot nematodes were two calcium-dependent ATPases, which are good candidates for playing a regulatory role in feeding-cell formation, potentially through cytoskeletal rearrangements.

Confocal microscopy of the plant cytoskeleton in response to nematodes was described in subsequent talks by Lieve Gheysen (University of Ghent, Belgium) and myself. Gheysen emphasized the recruitment of host cell-cycle control by invading nematodes. She outlined an interesting model for the steps leading to this recruitment, generated on the basis of her discovery of a nematode-encoded ubiquitin-extension protein that localizes to the nucleolus when transgenically expressed in plants. I presented genetic evidence that plants respond to a diffusible root-knot nematode signal via the same pathway used to respond to the Nod-factor signal produced by nitrogen-fixing rhizobacteria, and proposed a model whereby the root-knot nematode signaling molecule is functionally equivalent to Nod factor. This is consistent with my group's previous discovery by expressed sequence tag (EST) sequencing of Nod-factor biosynthesis enzymes in root-knot nematodes, which we postulated were acquired from rhizobia by HGT. Comparative genomics showed that the response pathway extends beyond the legumes, but intriguingly is absent from *Arabidopsis*, an observation that correlates with this species' poor status as a root-knot nematode host.

Dick Hussey (University of Georgia, Athens, USA) described results from studies of cDNA libraries made from micro-aspirated nematode pharyngeal glands, with a focus on secreted proteins, which are of especial interest for their potential role in the parasitic interaction. Although the number of genes found in the libraries is only a small sample of the estimated 5,000 or more secreted proteins, analysis of the approximately 50 genes from root-knot nematodes and the 70 genes identified in related cyst nematodes has revealed important candidates, including a ubiquitin-extension

protein, supporting Gheysen's work. Valerie Williamson (University of California, Davis, USA) and Isgouhi Kaloshian (University of California, Riverside, USA) presented back-to-back talks on host resistance to nematodes, aphids and white flies mediated by the *Mi* gene. Williamson emphasized the integration of *Mi* with other cellular machinery, largely on the basis of findings using assays in transgenic *Nicotiana benthamiana* leaves. In contrast, Kaloshian reported a genetic approach for identifying genes acting in consort with (and apparently upstream of) *Mi*, and has developed a tool for reverse genetics based on virus-induced gene silencing. Qingli Liu, one of Williamson's students, reported on the development of the diploid root-knot nematode *Meloidogyne hapla* as a genetic model, and remarkably the group appears to have identified and isolated from *Meloidogyne javanica* a gene required to elicit *Mi*-mediated resistance.

A significant number of talks emphasized interactions between nematodes and bacteria. In a symposium on biocontrol of nematodes, Charlie Opperman (North Carolina State University, Raleigh, USA) presented the nearly-completed genome sequence of the nematode parasitic bacterium *Pasteuria penetrans*. A deep phylogeny based on 40 orthologous housekeeping genes from 33 bacterial species placed *Pasteuria* in the *Bacillus* clade, but ancestral to other sequenced bacilli (including *Bacillus anthracis*). There is significant synteny across the clade and this provides a means to dissect biology. For example, the *Pasteuria* sequence revealed the presence of short collagens, which function in the spore coat and may be involved in host recognition. Remarkably, only the animal-pathogenic bacilli have these genes, and Opperman speculated that they may have been acquired by an ancient *Pasteuria* from insects or nematodes by HGT. In a separate symposium on nematode-bacterial associations, Opperman described an attack using nuclear magnetic resonance (NMR) on the regulation of the *Pasteuria* transition state from growing to sporulating cells. On the basis of comparative genomics across the bacilli, a role for certain metal ions in sporulation was predicted and experimentally confirmed *in vivo*. In that same session, Jonathan Hodgkin (Oxford University, UK) demonstrated the power of the *C. elegans* genetic and genomic system to reveal and then characterize the worm's innate immune response to bacterial pathogens. Strikingly, nematodes can distinguish many different bacteria and mount distinct and appropriate defense responses. As is the case with mammalian and insect innate immunity, the extracellular-signal-regulated protein kinase (ERK) and mitogen-activated protein (MAP) kinase cascade is involved, but in a unique manner in *C. elegans*. Elizabeth Scholl (North Carolina State University) presented a computational approach for examining HGT from bacteria to nematodes and made a compelling case for HGT as a driving force in nematode evolution. A key argument was that any newly transferred gene will experience strong ameliorative selective pressure, driving the gene toward characteristics of the host genome. Nevertheless, the idea of HGT remains controversial and was specifically challenged in a paper by undergraduate student Adler Dillman (Adams lab, Brigham Young University, Provo, USA) who, on the basis of analysis of differential selective pressure and alternative codon usage, questioned two of the 12 genes postulated by Scholl to have been acquired by HGT.

A workshop on nematode genomics was opened with the announcement by Opperman that the National Science Foundation (NSF) and United States Department of Agriculture (USDA) Interagency Microbial Genome Sequencing Program has funded a project to sequence the genome of *M. hapla*. The initial sequencing target is for five-fold coverage, but will be based on a minimum tile of BACs, generated via a five-enzyme, four-dye-based physical map. Opperman affirmed the project's commitment to rapid public dissemination of data, and invited community participation in the annotation and exploitation of the sequence data. As noted by Jim McCarter (Divergence Inc. and Washington University, St. Louis, USA), genome assembly for a human parasite, *Brugia malayi*, has proven unusually difficult, suggesting that the BAC-by-BAC strategy may prove prescient. Opperman also presented an initial draft of a physical map of *Meloidogyne incognita* and noted regions of unusual assembly, which he attributed to regions of highly repetitive sequence in this polyploid, parthenogenetic species. McCarter summarized the status of completed (*C. elegans* and *Caenorhabditis briggsae*) and in progress (*B. malayi* and three more Caenorhabditids) worm genomes and announced that *Trichinella spiralis* and *Pristionchus pacificus* genomes are now official National Human Genome Research Institute (NHGRI) projects, with the likely scenario of eight-fold sequence coverage and two rounds of automated finishing; I noted that a five-fold shotgun sequence of *Haemonchus contortus* has also recently been funded by the Wellcome Trust. Kelly Thomas (University of New Hampshire, Durham, USA) and I presented strategies to select 100 additional nematode species for whole-genome sequencing and committed to publishing a white paper in the *Journal of Nematology*. McCarter reported that more than half a million ESTs have been generated from 39 nematode species, yielding approximately 65,000 distinct genes, and reiterated the important point that community involvement and free and public availability of the data remain essential.

A 'Tree of Life' session opened with a presentation by Thomas about NemATOL [http://nematol.unh.edu], an online database for the nematode branch of the 'Assembling the Tree of Life' project that integrates morphological and 18S rRNA sequence data to create a searchable infrastructure that includes display of sequence alignments and phylogenies. David Fitch (New York University, USA) reported on a meeting of the group focusing on reconstructing the Rhabditina clade (which includes the genus *Caenorhabditis*) using a combination of ribosomal DNA and cDNA sequences (Figure 1). A broad phylogeny representing many taxa will be

constructed using small subunit sequences, and a subset of this large group of species will be represented in a better resolved tree based on large subunit sequences. Deeper branches will be explored with cDNA sequences. Adams led a discussion on resolution of the Tylenchina, which include the family Heteroderidae and the genus *Meloidogyne*. The identification of missing taxa and the determination of genes for improved resolution of deep nodes remain major open issues for this section of the nematode phylogeny. Sergei Subbotin (University of California, Riverside) demonstrated a method for the creation of conservative alignments for 28S rRNA by removing ambiguous regions, thus reducing the subjectivity of alignments for phylogenetic reconstruction. Jim Baldwin (University of California, Riverside, USA) discussed the importance of meaningful classification to better reflect phylogenetic relationships. The recent paper by Rokas *et al*. (*Nature* 2003, **425:**798-804) analyzing differences between phylogenies based on individual genes was introduced by Virginia Ferris (Purdue University, West Lafayette, USA) in conjunction with a discussion on the need to analyze multiple genes to reduce incongruencies in phylogenetic reconstruction. She also spoke of the current problems regarding differentiation between orthologs and paralogs. In response, Scholl described her recent efforts to establish clusters of orthologous groups based on EST data for use in a multi-gene phylogeny of eight species of Heteroderidae. And the session ended with the introduction of a 'wish list' for biological material or rDNA sequences of nematode species that are considered key to future phylogenetic studies, and the identification of attendees who may be able to contribute biological materials for nematodes for which no ribosomal sequences currently exist.

Have we taken our blinders off? The answer is a resounding yes. Genomics has empowered research on what in the past have been 'fringe' organisms to the point that diverse nematodes are now serving as potent models to address broad questions in biology. Abstracts can be searched online by keyword [http://www.nematologists.org/annualmeeting/searchabstracts.ace] and will be published in the *Journal of Nematology*, 2004.

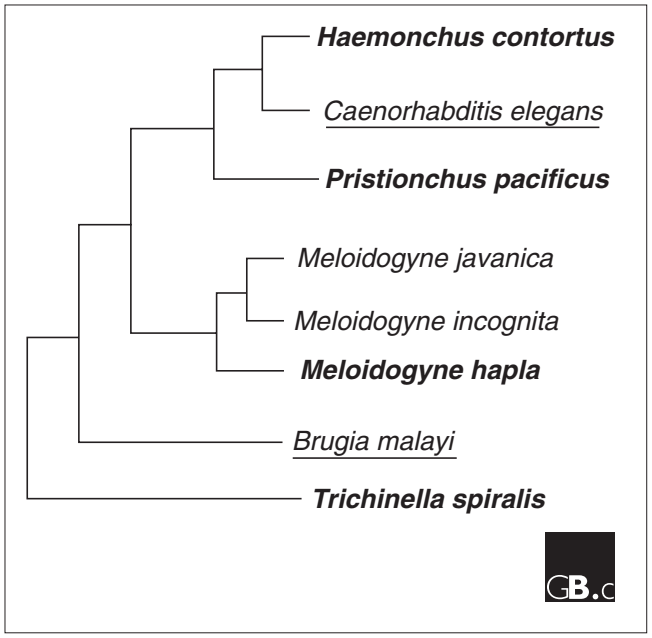

Figure 1
Schematic representation of the relationships between some of the nematodes mentioned in this report. Underlined entries indicated completed genomes (more than eight-fold coverage), and those in bold are ongoing, funded projects. The branch lengths are arbitrary.