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Research Focus

Caenorhabditis evolution: if they all look alike, you aren't looking hard enough

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Caenorhabditis elegans **is widely known as a model organism for cell, molecular, developmental and neural biology, but it is also being used for evolutionary studies. A recent meeting of researchers in Portugal covered topics as diverse as phylogenetics, genetic mapping of quantitative and qualitative intraspecific variation, evolutionary developmental biology and population genetics. Here, we summarize the main findings of the meeting, which marks the formal birth of a research community dedicated to** ***Caenorhabditis*** **species evolution.**

Introduction

The nematode *Caenorhabditis elegans* has many virtues as a research organism. These include having a short generation time and reproducing by both selfing and outcrossing, in addition to simplicity of culture in the laboratory, ability to be studied using advanced forward and reverse genetics, having the first sequenced animal genome, and the strong tradition of cooperation among 'worm people'. These advantages enabled *C. elegans* to become a leading model for molecular biology and genomics by the 1990s. Its attributes are also increasingly attracting the attention of evolutionary biologists, who recognize that the deep understanding of *C. elegans* can be a sturdy foundation for comparative biology. This recognition has its roots in the descriptions of reproductive variation in the family Rhabditidae by Emile Maupas [1] and Hikokuro Honda [2], and later by Victor Nigon and Ellsworth Dougherty [3], who compared the anatomy of, and examined reproductive isolation between, *C. elegans* and *Caenorhabditis briggsae*.

Since these early studies, the number of researchers dedicated to evolutionary analysis of *Caenorhabditis* species has grown steadily. In May 2006, almost all of these researchers met in Oeiras, Portugal, to celebrate recent successes, to ponder new breakthroughs and to establish formally a research community that will continue to meet every two years. Hosted by the Instituto Gulbenkian de Ciência, and generously supported by the European Molecular Biology Organization (EMBO), the 57 participants of the workshop addressed a wide range of topics. Some of these (e.g. molecular systematics and sex determination) are well established areas of research. Others (e.g. the population genetics of natural isolates and the genetic mapping of intraspecific variation) are emerging areas with enormous potential. Here, we describe the main findings that were presented at the workshop, with emphasis on these emerging areas (for further details, see Ref. [4]).

Phylogeny

The phylogenetic relationships of *C. elegans* to its rhabditid relatives, which are being determined as part of the Assembling the Tree of Life program (funded by the National Science Foundation), were presented by Karin Kiontke and David Fitch (New York University). The newly refined phylogenetic tree places *C. elegans* in the middle of a large and diverse group of rhabditid nematodes and demonstrates that several features have evolved convergently [5]. For example, hermaphroditism has evolved at least nine times from gonochoristic ancestors (i.e. from ancestors with separate male and female sexes), but there is only one probable case of hermaphroditism reverting to gonochorism. Certain features of male tail anatomy have altered in a convergent manner, and studies are underway to determine whether the underlying mechanisms are similarly convergent. Ultimately, study of these replicated evolutionary events should provide insight into the extent to which genetic, genomic and developmental systems constrain evolution.

Genomics tools for species other than *C. elegans*

The genomes of *C. elegans* [6] and *C. briggsae* [7] were the first pair of closely related animal genomes to be sequenced, and these organisms are a fantastic tool for evolutionary studies. At the workshop, much excitement revolved around the sequencing of three more species, by the Genome Sequencing Center at Washington University in St. Louis (WUGSC): *Caenorhabditis remanei*, *Caenorhabditis* n. sp. 4 (represented by strains CB5161 and PB2801, among others) and *Caenorhabditis japonica*.

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Box 1. Websites for exploring *Caenorhabditis* species diversity and comparative genomics

Natural isolates

- http://www.cbs.umn.edu/CGC/
  The *Caenorhabditis* Genetics Center (CGC) at the University of Minnesota provides many isolates of *Caenorhabditis elegans* and other *Caenorhabditis* species for free.
- http://www2.ijm.jussieu.fr/worms/
  The laboratory of Marie-Anne Félix, at the Institut Jacques Monod, has an extensive collection of natural isolates of *Caenorhabditis* species, many unavailable from the CGC.
- http://www.nyu.edu/projects/fitch/WSRN/
  David Fitch of New York University maintains the Worm Systematic Resource Network, a database of available nematode strains with an emphasis on the family Rhabditidae, which includes several species of *Caenorhabditis*.

Comparative genetics and genomics

Caenorhabditis briggsae

- http://www.wormbase.org
  The most up-to-date annotation of the *C. briggsae* genome is embedded throughout WormBase for *C. elegans* researchers, most obviously in the form of ortholog predictions and syntenic alignments. It can also be analyzed separately by download links.
- http://wormlab.caltech.edu/briggsae/
  Bhagwati Gupta of McMaster University maintains a site for researchers working intensively with *C. briggsae*.
- http://snp.wustl.edu/snp-research/c-briggsae/index.html
  Draft *C. briggsae* DNA polymorphism maps being produced by Raymond Miller and colleagues at the Genome Sequencing Center at Washington University in St. Louis are available at this website.

Caenorhabditis remanei

- ftp://dev.wormbase.org/pub/wormbase/genomes/remanei
  C. remanei preliminary gene predictions recently became part of WormBase homology summaries for *C. elegans* and *C. briggsae* genes. The current draft assembly of the *C. remanei* genome and preliminary gene predictions are available from this site.
- http://dev.wormbase.org/db/seq/gbrowse/remanei/
  WormBase has also provided a preliminary web browser for the *C. remanei* genome.

Meetings

- http://cwp.embo.org/w06-31/
  EMBO Workshop on The Study of Evolutionary Biology with *Caenorhabditis elegans* and Closely Related Species (May 2006; Oeiras, Portugal)
- http://www.union.wisc.edu/celegans/index.html
  C. elegans Development & Evolution Topic Meeting #1 (June 2006; Madison, Wisconsin)

The project aims to produce 9 × genome coverage of each using a whole-genome shotgun sequencing approach. A preliminary assembly and browser for *C. remanei* are available from the WormBase database (Box 1) and have already proved valuable for the identification and comparison of orthologous genes [8]. A preliminary assembly for *Caenorhabditis* n. sp. 4 (strain PB2801) will be released soon (J. Spieth, personal communication), and *C. japonica* DNA entered the sequencing pipeline in January 2007. An isolate from China that was recently identified as a new sister species to *C. briggsae, Caenorhabditis* sp. 5 JU727 (M-A. Félix, unpublished), was suggested, at the workshop, to be an interesting candidate for a genome-sequencing project in the future.

The first set of gene predictions for the preliminary *C. remanei* assembly is unexpectedly large (J. Spieth, personal communication): ~26 000, in contrast to 20 000 for both *C. elegans* and *C. briggsae*. This gene set will be refined by more sequencing and expressed-sequence tag (EST) reads by the WUGSC. The improved gene set should clarify why *C. remanei* seems to have ~30% more genes than expected. Interestingly, using flow cytometry, the genome size of three gonochoristic species (*C. remanei, C. japonica* and *Caenorhabditis* n. sp. 4) is directly estimated to be ~135 Mb (J.S. Johnston and R. Waterston, personal communication), again larger than the genome of *C. elegans* and *C. briggsae*. Placed in a phylogenetic context, this difference in size suggests that the shift to selfing coincides with a significant reduction in both DNA and gene content, but the details of this change await definitive genome assemblies. For the *C. remanei* genome, the assembly will be facilitated by the integration of additional sequence reads and by the resolution of potential residual heterozygosity in the sequenced strain.

A key experimental tool for any organism is a high-resolution genetic map tied to the genome sequence. Matthew Rockman (Princeton University) and Dan Koboldt (Washington University in St. Louis) presented newly developed dense genetic polymorphism maps for *C. elegans* and C. *briggsae,* respectively, both of which were made by genotyping single-nucleotide polymorphisms (SNPs) in recombinant inbred lines generated from divergent natural isolates. The *C. briggsae* genetic map and more than 200 000 SNPs are available (Box 1), and a new *C. briggsae* genome assembly based on this map will be available soon from WormBase.

In addition to enabling improved gene predictions, sequencing multiple *Caenorhabditis* species genomes should help to identify regulatory elements more accurately[*]. Erich Schwarz (California Institute of Technology) presented promising results from using the software Cistematic (http://cistematic.caltech.edu) for identifying putative *cis*-regulatory elements conserved among *C. elegans*, *C. briggsae* and *C. remanei.* Another useful resource for researchers will be a database of orthologs between, and paralogs in, the five *Caenorhabditis* species. Avril Coghlan (Wellcome Trust Sanger Institute) gave a presentation about TreeFam (http://www.treefam.org), a database of phylogenetic trees of animal gene families that is now fully integrated into WormBase gene reports [9]. At present, TreeFam includes *C. elegans*, *C. briggsae* and *C. remanei* genes, and, from the phylogenetic trees, it infers orthologs between, and paralogs in, these three species.

Evolution of development and anatomy

Caenorhabditis species have markedly similar anatomy and development, despite genome-sequence divergence that is greater than that seen among all vertebrates [10]. However, with careful observation, anatomical differences are being discovered and dissected. Research presented at the workshop by Helen Chamberlin (The Ohio State University) and Scott Baird (Wright State

[*]Sternberg, P. *et al.* (2003) Genome sequence of additional *Caenorhabditis* species: enhancing the utility of *C. elegans* as a model organism. (http://genome.wustl.edu/ancillary/data/whitepapers/Caenorhabditis_WP.pdf)

University) focused on the developmental and anatomical differences in the excretory system and in the rays (peripheral sensory organs) of the male tail. In the excretory system, a single gene (*lin-48*) has a particularly large effect. Expression of *lin-48* in the excretory duct cell of *C. elegans* confers specific anatomical features and higher salt tolerance on this species than on others in the genus [11,12]. By contrast, multiple genes contribute to differences in male ray anatomy [13]. Complementing these studies, the research groups of Marie-Anne Félix (Institut Jacques Monod) and Bhagwati Gupta (McMaster University) identified surprising differences in the development of the vulva, which is anatomically conserved, and Scott Emmons (Albert Einstein College of Medicine) described unexpected variation in the genes regulating the Hox complex.

The most obvious variable trait that distinguishes *Caenorhabditis* species is the reproductive mode: most are gonochoristic, but *C. elegans* and *C. briggsae* use a derived form of self-fertile hermaphroditism. In both of these species, the key to selfing is the generation of sperm in the chromosomally female germ line. Eric Haag (University of Maryland), Dave Pilgrim (University of Alberta) and Ronald Ellis (University of Medicine & Dentistry of New Jersey) compared the germ-line sex-determination pathway of *C. elegans* with those of *C. briggsae* and *C. remanei*. (All three research groups are moving beyond using RNA interference to produce true mutations in non-*C. elegans* species [14].) Their results indicate that the hermaphroditism of *C. elegans* and *C. briggsae*, although overtly similar, evolved by distinct modifications of the ancestral sex-determination pathway. Also addressing mating-system evolution, King Chow (The Hong Kong University of Science and Technology) described a male-attracting pheromone that is produced by *C. remanei* females but seems to have been lost by hermaphroditic species.

Experimental evolution

Another emerging area is the use of laboratory evolution experiments to test models of phenotypic evolution. Until recently, the main examples of this approach have been experiments using mutation-accumulation strains to explore the consequences of deleterious mutation. Dee Denver (Oregon State University) and W. Kelley Thomas (The University of New Hampshire) described the latest results from a several-hundred-generation mutation-accumulation experiment. Recent analyses [15] of the effects of mutation on gene expression are particularly interesting, because they suggest that there is a substantial amount of stabilizing selection on gene expression across the genome. Researchers have started using experimental evolutionary approaches to directly study models of adaptive evolution. Andrew Peters (University of Wisconsin–Madison) showed rapid recovery of fitness in knockout strains of *C. elegans* allowed to adapt over 100 generations; Henrique Teotónio, Sara Carvalho (both of the Instituto Gulbenkian de Ciência) and Patrick Phillips (University of Oregon) showed that aspects of the breeding system (e.g. the frequency of males under androdioecy) respond to selection in the laboratory [16]. Even coevolution of species is being addressed by experimental approaches: Rebecca Schulte and Hinrich Schulenburg (Universität Tübingen) presented a promising coevolutionary model system that uses *C. elegans* and its parasite *Bacillus thuringiensis*.

Intraspecific variation and population genetics

Although, ultimately, evolution proceeds through population processes, nearly all *C. elegans* studies in the literature are based on the strain Bristol N2. The first thorough analysis of natural phenotypic variation, by Jonathan Hodgkin and Tabitha Doniach [17], revealed substantial differences in fecundity, fertility, mating behavior and spontaneous production of males. Since then, around a dozen published studies have shown considerable variation among *C. elegans* isolates (e.g. in foraging and aggregation behavior [18], longevity [19], chemosensory behavior [20] and microbial pathogen susceptibility [21,22]). Work presented at the workshop showed that various researchers are actively mapping the genes controlling this variation, using the quantitative trait locus (QTL) approach. Simon Harvey (University of Bristol) has mapped plasticity in the formation of dauer larvae, and Jan Kammenga (Wageningen Universiteit) reported an impressive example of QTL mapping, in which variation in the calpain-family-protease-encoding gene *tra-3* (mainly known for its role in sex determination) was implicated in temperature-dependent plasticity of body size. Matthew Rockman also reported progress in using his *C. elegans* map to clone the copulatory plug formation locus, *plg-1*, which is naturally variable.

These results for phenotypic variation emerged in parallel with studies of genetic variation [23–26]. Jody Hey (Rutgers), Antoine Barrière (Institut Jacques Monod), Matthew Rockman, Michael Ailion (The University of Utah), and Elie Dolgin and Asher Cutter (both of The University of Edinburgh) collectively produced an exciting new model of the genetic structure of natural *C. elegans* populations. In this model, completely homozygous strains have widespread distribution and are often found together in close proximity. Given that the life of hermaphrodites largely involves selfing, this might be expected if rare males also mate poorly, as was predicted for the Bristol N2 strain by earlier studies [27,28]. However, field studies reveal a surprisingly high frequency of heterozygotes, and linkage disequilibrium between strains, although high, is not absolute. These seemingly incongruous observations are reconciled by the demonstration of strong outbreeding depression, including F2 lethality, between different homozygous strains. Overall, it seems that each homozygous genotype is 'locally adapted' to itself by a unique combination of epistatic factors and that, despite fairly frequent hybridization events, the genotypes retain their distinctiveness owing to hybrid inviability. These results are in contrast to those for *Arabidopsis thaliana*, another model species that is highly selfing, which shows evidence of significant local adaptation and genetic isolation-by-distance in different strains (or 'ecotypes') [29].

Concluding remarks

This first workshop made it abundantly clear that *Caenorhabditis* researchers from both organismal and

suborganismal areas of biology have much to learn from each other. The group unanimously agreed that continuing to meet every two years is essential, and the next meeting is now set to take place in Madison, Wisconsin, from 10 to 15 June, 2008. Mark your calendars!

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